OFFICIAL

METHODS OF ANALYSIS

OF THE

ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS

METHODS OF ANALYSIS
A. O. A. C.

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METHODS OF ANALYSIS

OF THE

ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS

NINTH EDITION, 1960

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Preface to Ninth Edition

THE AOAC

The Association of Official Agricultural Chemists, more familiarly known as the AOAC, is the professional organization of State and Federal scientists devoted to developing, testing, and approving methods for the analysis of fertilizers, feeds, pesticides, foods, drugs, cosmetics, caustic poisons, and other materials related to agricultural pursuits. It was organized in 1884 by the State and Federal chemists who were in charge of enforcement of State fertilizer laws, or who, as members of State agricultural experiment stations or the U. S. Department of Agriculture, were interested in practical and scientific applications of fertilizers to growing crops. Four meetings prior to 1884 had failed to produce a cohesive and stable organization of both official and commercial chemists. This final organizational form was settled upon with the full cooperation and even insistence of commercial chemists. They agreed upon the principle that since official chemists had the responsibility for enforcement of laws, they also had the responsibility for the choice of valid methods of analysis for this purpose. Voting is restricted to official chemists, but discussion is open to all chemists.

All state chemists, including those of universities and experiment stations, are members of the Association. Federal organizations represented in the Association are: Food and Drug Administration and Public Health Service of the Department of Health, Education, and Welfare; Department of Agriculture; Department of Defense; Internal Revenue Service of the Treasury Department; National Bureau of Standards of the Department of Commerce; and Fish and Wildlife Service, of the Department of Interior.

As the regulatory control of other commodities such as foods, feeds, drugs, cosmetics, and pesticides became a recognized governmental function, the work of the Association expanded. It has accepted the responsibility for providing the regulatory scientist with the accurate and reproducible methods of analysis that are required for the enforcement of laws and regulations, or for research on agricultural products. In fulfilling this responsibility the Association also meets the needs of the chemist in industry for methods upon which he can rely for testing commodities before they come within the jurisdiction of various laws. This is accomplished through adherence to a fundamental constitutional requirement that methods, to be approved by the Association, must be subjected to collaborative study. In such a study a number of chemists analyze the same samples by a proposed method in order to determine its accuracy and reproducibility in several laboratories. At the present time about 300 chemists, designated as "Associate Referees," are studying methods of analysis grouped in about 50 general categories from "Agricultural Liming Materials" to "Waters." Not all of them are official chemists; many are industry chemists who, because of their specialized knowledge and experience, also participate in the development and testing of methods of analysis. The results of the studies of these Associate Referees form the basis for the actions of the Association in its approval of methods.

The laws enforced by the AOAC members require objective, scientific evidence for their successful application. The AOAC attempts to provide for both industry and government a common meeting ground for discussion, at a scientific level, of the methods of analysis that will be used to obtain this evidence. That this has been successful is indicated by the facts that some state laws require the use of AOAC methods, where applicable; the Federal Definitions and Standards of Identity for many foods incorporate AOAC methods into their requirements; many Federal specifications and private contracts utilize AOAC methods; and AOAC methods have been quite generally accorded a preferred status in court testimony. Most important of all, the general recognition of AOAC methods removes from the realm of controversy the scientific question of relative validity and merits of the available methods of

analysis. This is settled by the scientists themselves on the basis of facts developed during their collaborative studies.

An equally important area of applicability of AOAC methods is in the field of composition and utilization of agricultural commodities. Numerous publications dispense with a description of their analytical procedures by reference to AOAC methods. Where the results of research are such that the analytical data are, in themselves, of interest and are to be compared with the data from other laboratories or where they are to be compiled into reference tables, then the validity and the reproducibility of the analytical methods used become an important factor in the value and significance of the research data. For example, the value of tables of food composition which include data for caloric value, protein, carbohydrate, fat, vitamin, and mineral contents depends directly upon the availability of standardized methods for determining each of these categories.

The results of 76 years of work by the Association are embodied in its primary publication, *Official Methods of Analysis of the Association of Official Agricultural Chemists,* now in its ninth edition. It is supplemented by the quarterly Journal which contains the transactions of the Association, including the annual changes in methods adopted by the Association, the reports of the referees, and contributed papers containing new methods, new applications, and authentic or interpretive data.

The Association deals only with methods of analysis and it maintains formal and informal cooperative arrangements with other scientific societies in order to maintain uniformity of methods of analysis. Among these are The American Public Health Association (*Standard Methods for the Examination of Dairy Products*), American Oil Chemists' Society (fats and feeds), American Society of Brewing Chemists (malt beverages), Joint Committee on Uniformity of Methods for Water Analysis (waters), and American Society for Testing Materials.

THE NINTH EDITION

As new methods are constantly being adopted, the number of pages has increased with each edition. Previously the Association managed to control the size of the book by a system of abbreviating words and omitting articles and prepositions. In the ninth edition, however, the volume of new material has outstripped the Association's ability to devise new abbreviations and omissions, and in order to keep the book within a single volume, the format has been radically revised to a larger page size containing two columns of type.

The chapter on soils has been omitted, reflecting the Association's decision that the requirements of methods in this field could be provided more appropriately by a specialized society. The classical scheme of elementary analysis formerly in the chapter on soils has been retained in the chapter on liming materials.

Two large chapters have been subdivided into logical divisions. The bacteriological methods for disinfectants have been separated from the chemical methods for pesticides, and now constitute a distinct chapter. Likewise, the specific methods for drugs in feeds, which emphasize the determination of small amounts of active components in a large bulk of food material, have been segregated from the primary therapeutic agents for human and veterinary use, and placed in a separate chapter.

Many new methods consist of separating the desired component by chromatography and then determining it colorimetrically. For example, the sugars of honey are separated on a charcoal column, and although the individual sugars are determined by classical volumetric techniques, the efficiency of the separation is monitored by paper chromatography. Paper chromatographic techniques are included in this edition, for the first time, to detect commercial glucose in honey, differentiate technical benzene hexachloride from lindane, and detect foreign botanicals in vanilla extract.

Chromatographic techniques have been used to advantage in nearly every field of active methods development. In fact, virtually all successful analytical methods for minute amounts (parts per million) of pesticide residues depend on the ability of chromatographic columns to differentiate between the wanted and unwanted components of highly complex mixtures.

As a consequence of fundamental studies sponsored by the Flavoring Extracts Manufacturers Association, a paper chromatographic method has been adopted for the detection of adulteration of vanilla extracts, and other methods are in process of development. This study is significant because it demonstrates the

realization by a group representing an industry that regulatory agencies do not have adequate facilities to develop all the methods required for consumer protection and industry control.

Examples of progress in development of methods of analysis in other areas include a photometric method for phosphorus in fertilizers, a radioactive tracer method using Cl^{36} for the determination of benzene hexachloride in pesticide formulations, and an infrared method for the identification of gums in foods. An international collaborative study, under the auspices of the International Union of Pure and Applied Chemistry, has led to an improved method for the determination of trace amounts of copper. For physiologically active materials, bioassay often remains the only practicable method of analysis. New methods of this kind include a mouse test for paralytic shellfish poison, a microbiological assay for antibiotics in feed supplements, and a biological method for determining protein quality. The microbiological methods for the B vitamins have been completely revised to form a single unified method with appropriate modifications for the individual vitamins.

The eighth edition of *Official Methods of Analysis* was published shortly after the enactment of the pesticides amendment to the Federal Food, Drug and Cosmetic Act, the enforcement of which required that an analytical method be available for the determination of the amount of a given pesticide residue remaining on a treated crop. This amendment has resulted in the promulgation of more than 2000 tolerances or exemption from tolerances for more than 100 pesticide chemical residues on specific "raw agricultural commodities." Residues above these tolerances reflect a potential danger to public health which must be policed through suitable analytical methods.

The previous edition contained methods for only two of the newer organic pesticides—DDT and parathion. Of the limited number of methods tested by the AOAC collaborative procedure, only eight—Aramite, benzene hexachloride, captan, malathion, methoxychlor, piperonyl butoxide, Sulphenone, and tetramethylthiuram disulfide—have been found suitable for adoption by the AOAC during the past five years. In some cases the method is applicable only to a few crops. More time and facilities must be made available for methods research and testing before adoption of methods can hope to keep pace with the promulgation of regulations.

The food additives amendment to the Federal Food, Drug and Cosmetic Act, passed by the Congress of the United States in 1958, requires that a petitioner seeking authorization for the use of a new food additive shall furnish "a description of practicable methods for determining the quantity of such additive in or on food, and any substance found in or on food because of its use." The interpretive regulations issued under this amendment require that the petitioner include in his petition "a description of practicable methods to determine the amount of food additive in the raw, processed and/or finished food and of any substance formed in or on such food because of its use. The test proposed shall be one that can be used for food-control purposes and that can be applied with consistent results by any properly equipped and trained laboratory personnel."

The Deputy Commissioner of the Food and Drug Administration, in discussing this requirement, commented: "The very essence and key to the success and effectiveness of this kind of legislation lies every bit as much in the development of suitable methods for determining quantitatively as well as qualitatively the presence of residues in or on food as provided for by the regulation as in the development of the all-important toxicity data showing that the amount is safe. Without such precise methodology, the effort and expense of developing toxicity data will go for naught."

Recognizing that there cannot be inflexible test requirements in these fields, and that each case must be judged individually, the Referee on Metals, Other Elements, and Residues in Foods has suggested the following minimum requirements for reliability and sensitivity:

Reliability: Provision for an adequate reference standard; an accuracy of $\pm 10\%$ at the tolerance level and sensitivity to about 10% of such level; a food blank that does not exceed 10% of the tolerance; and reproducible correction factors, if present. Generally, as the tolerance level decreases, some relaxation of these criteria may be realistically indicated.

Specificity: Applicability at the tolerance level in the presence of the normal components of the foods and in the presence of any other chemicals, including other food additives or pesticide residues, that can reasonably be expected to be present in such foods.

"In brief, a practicable method is one which is adequate to enable a regulatory agency to take action against a shipment of food containing a food additive or a pesticide residue in amounts exceeding the safe tolerance, thus protecting the public health." The evaluation of methods of analysis for constituents which may be present at levels of parts per million obviously requires a scale of values far different from that which is appropriate for constituents that may be present at levels of parts per hundred.

THE METHODS

Users who are familiar with previous editions will find no substantial changes in style or arrangement. The attention of new users should be called to the necessity for consulting the Definition of Terms and Explanatory Notes for the style conventions used throughout the volume. Nearly all articles and many prepositions are omitted. Cross references for methods, apparatus, and reagents are used extensively.

Methods are designated as "Official," "First Action," and "Procedures." A method which, after an initial collaborative study, shows a suitable degree of accuracy and reproducibility, is adopted as *first action.* This status provides an opportunity for those who are interested in the method to study it further before its final adoption as *official.* A few well-established types of examinations, such as sampling and preparation of sample, which are difficult to study collaboratively, are designated *procedures,* but under appropriate conditions they also may be adopted as official methods.

New methods and modifications of established methods are adopted at each annual meeting of the Association. These changes in methods are published in the Journal of the AOAC and may be obtained separately by subscription.

ACKNOWLEDGMENTS

The Journal of the AOAC contains the record of those who have made this vast compilation possible—the collaborators, associate referees, referees, committee members, and officers of the Association. Only through their continued cooperation is it possible to provide this unrivaled treatise of tested analytical methods.

William Horwitz
Secretary Treasurer
Association of Official
Agricultural Chemists, Inc.

Washington, D. C.
May 31, 1960

Abstract from

Preface to First Edition

"In presenting this revision of the official and tentative methods of analysis of the Association of Official Agricultural Chemists, it is appropriate to give a brief statement of the organization of the Association, its purpose, and the procedure by which the methods are adopted.

"Membership in the Association is institutional and includes the State Departments of Agriculture, the State Agricultural Colleges and Experiment Stations, the Federal Department of Agriculture, and the Federal, State, and City offices charged with the enforcement of food, feed, drug, fertilizer, insecticide and fungicide control laws.

"The Association was founded at Philadelphia, Pa., September 9, 1884, by the following representative agricultural chemists of that time, the organization being the result of a series of informal meetings held the immediately preceding years:

"Prof. H. W. Wiley, Chemist of the Department of Agriculture, Washington, D. C.

Mr. Clifford Richardson, Assistant Chemist of the Department of Agriculture, Washington, D. C.

Mr. Philip E. Chazal, State Chemist of South Carolina.

Dr. Chas. W. Dabney, Jr., State Chemist of North Carolina.

Dr. W. J. Gascoyne, State Chemist of Virginia.

Dr. E. H. Jenkins, Connecticut Experiment Station.

Prof. John A. Myers, State Chemist of Mississippi.

Prof. H. C. White, State Chemist of Georgia.

Mr. C. DeGhequier, Secretary National Fertilizer Association.

Dr. Schumann, Dr. Lehmann, Mr. Gaines and others."

Contents

Illustrations

Definitions of Terms and Explanatory Notes

(1) The term "H_2O" means distilled water, except where otherwise specified, and except where the water does not mix with the determination, as in "H_2O bath."

(2) The term "alcohol" means 95% ethyl alcohol. Alcohol of strength x% may be prepared by diluting x ml 95% alcohol to 95 ml with H_2O.

(3) The term "ether" means ethyl ether that is peroxide-free by following test: To 10 ml of the ether in small, clean g-s. cylinder previously rinsed with the ether, add 1 ml freshly prepd 10% KI soln. Shake, and let stand 1 min. No yellow color should be observed in either layer.

(4) The reagents listed below, unless otherwise specified, have approx. strength stated and conform in purity with Recommended Specifications for Analytical Reagent Chemicals of the American Chemical Society:

	Assay
Sulfuric acid	95.0–98.0% H_2SO_4
Hydrochloric acid	36.5–38.0% HCl
Nitric acid	69.0–71.0% HNO_3
Fuming nitric acid	Not <90% HNO_3
Acetic acid	Not <99.7% $HC_2H_3O_2$
Hydrobromic acid	47.0–49.0% HBr
Ammonium hydroxide	28–30% NH_3
Phosphoric acid	Not <85% H_3PO_4

(5) All other reagents and test solns, unless otherwise described in text, conform to requirements of American Chemical Society. Where such specifications have not been prepd, use highest grade reagent. When anhyd. salt is intended, it is so stated; otherwise the crystallized product is meant.

(6) Phenolphthalein (phthln) used as indicator is 1% alc. soln unless otherwise specified; methyl orange is 0.1% aq. soln.

(7) Directions for standardizing reagents are given in Chapter **42**.

(8) Unusual reagents not mentioned in reagent sections or cross referenced, other than common reagents normally found in the laboratory, are italicized the first time they occur in a method.

(9) In expressions (1+2), (5+4), etc., used in connection with name of reagent, first numeral indicates vol. of reagent used, and second numeral indicates vol. of H_2O. For example, HCl (1+2) means reagent prepd by mixing one vol. of HCl with two vols of H_2O. When one of reagents is solid, expression means parts by wt, first numeral representing solid reagent and second numeral H_2O.

(10) In making up solns of definite percentage it is understood that x g of substance is dissolved in H_2O and dild to 100 ml. Although not theoretically correct, this procedure will not result in any appreciable error in any of methods given in this book.

(11) All calcns are based on table of international atomic weights, **43.001.**

(12) Vol. flasks and pipets conform to specifications of the National Bureau of Standards as described in NBS Circular 602, April 1, 1959.

(13) Sieve designations, unless otherwise specified, are those of National Bureau of Standards as described in Federal Specification RR-S-366B, July 27, 1953, and Amendment 1, June 4, 1958. The designation " '100-mesh' (or other number) powder (material, etc.)" means material ground to pass thru standard sieve No. 100 (or other number).

(14) The term "paper" means filter paper, unless otherwise specified.

(15) The term "high speed blender" designates a mixer with 4 canted, sharp-edge, stainless steel blades rotating at the bottom of a 4-lobe jar at 10,000–12,000 rpm. Suspended solids are reduced to fine pulp by action of the blades and the lobular shape of the container which swirls the suspended solids into the blades. Waring Blendor, or equiv., meets these requirements.

(16) A "flat-end rod" is a glass rod with one end flattened by heating to softening in flame and pressing vertically on flat surface to form circular disk with flat bottom at the end.

(17) Unless otherwise indicated, temps are expressed as degrees Centigrade.

(18) Operations specified as "wash (rinse, ext., etc.) with two (three, four, etc.) 10 ml (or other quantity) portions H_2O (or other solvent)" mean that the operation is to be performed with the indicated vol. of solvent and repeated with the same vol. of solvent until number of portions required have been used.

(19) Definitions of terms used in methods involving spectrophotometry are those given in *J. Assoc. Offic. Agr. Chemists* **37**, 54(1954). The most important principles and definitions are:

(**a**) More accurate instrument may be substi-

tuted for less accurate instrument (e.g., spectrophotometer may replace colorimeter) where latter is specified in method.

(b) *Absorbance(s)* (*A*).—Negative logarithm to base 10 of ratio of transmittance of sample to that of reference or std material. Other names that have been used for quantity represented by this term are optical density, extinction, and absorbancy.

(c) *Absorptivity(ies)* (*a*).—Absorbance per unit concn and cell length. $a = A/bc$, where b is in cm and c in g/L, or $a = (A/bc) \times 1000$, if c is in mg/L. Other names that have been used for this or related quantities are extinction coefficient, specific absorption, absorbance index, and $E^{1\%}_{1cm}$.

(d) *Transmittance(s)* (*T*).—Ratio of radiant power transmitted by sample to radiant power incident on sample, when both are measured at same spectral position and with same slit width. Beam is understood to be parallel radiation and incident at right angles to plane parallel surface of sample. If sample is soln, solute transmittance is quantity usually desired and is detd directly as ratio of transmittance of soln in cell to transmittance of solvent in an equal cell. Other names that have been used for this quantity are transmittancy and transmission.

(e) Standardization.—Spectrophotometer may be checked for accuracy of wavelength scale by reference to Hg lines: 239.94, 248.27, 253.65, 265.37, 280.4, 302.15, 313.16, 334.15, 365.43, 404.66, 435.83, 546.1, 579.1, 623.44, 671.62, and 690.72 mμ. To check consistency of absorbance scale prep. soln of 0.0400 g K_2CrO_4/L 0.05*N* KOH and det. absorbance at following wavelengths in 1 cm cell (J. Research NBS, **48,** 414 (1952)): 230 mμ, 0.171; 275, 0.757; 313.2, 0.043; 375, 0.987.

(20) *Perchloric acid.*—Contact of $HClO_4$ soln with oxidizable or combustible materials or with dehydrating or reducing agents may result in fire or explosion. Persons using this acid should be thoroly familiar with its hazards, and safety practices should include following:

(a) Remove spilled $HClO_4$ by immediate and thoro washing with large quantities of H_2O.

(b) Hoods and ducts for $HClO_4$ vapor should be made of chemically inert materials and so designed that they can be thoroly washed with H_2O. Exhaust system should discharge in safe location, and fan should be accessible for cleaning.

(c) Avoid use of org. chemicals in hoods employed for $HClO_4$ digestions.

(d) Use goggles, barrier shields, and other devices as may be necessary for personnel protection.

(e) In wet combustions with $HClO_4$ treat sample first with HNO_3 to destroy easily oxidizable org. matter.

(f) Contact of $HClO_4$ soln with strong dehydrating agents such as P_2O_5 or concd H_2SO_4 may result in formation of explosive anhyd. $HClO_4$. Exercise special care in performing analyses requiring the use of $HClO_4$ with such agents.

(g) Observe also the precautions outlined in: (1) "Perchloric Acid Solution," Chemical Safety Data Sheet SD-11 (1947), Manufacturing Chemists Association of the United States, 1825 Conn. Ave., N.W., Washington 9, D.C.; (2) "Applied Inorganic Analysis," W. F. Hillebrand, G. E. F. Lundell, H. A. Bright, and J. I. Hoffman, 2nd Ed., 1953, pp. 39–40, John Wiley and Sons, Inc., New York; (3) "Notes on Perchloric Acid and Its Handling in Analytical Work," *Analyst* **84,** 214–216 (1959).

(21) For sake of simplicity the abbreviations Cl and I instead of Cl_2 and I_2 are used for chlorine and iodine. Similar abbreviations have been used in other cases. The same abbreviation may also be used for the ion where no ambiguity will result.

(22) Sources of frequently referenced materials are:

ATCC: American Type Culture Collection, 2029 M St., N.W. Washington 6, D. C.

Eastman organic chemicals: Eastman Organic Chemicals Department, Distillation Products Industries, Rochester 3, N. Y.

NBS: National Bureau of Standards, Washington 25, D.C.

USP: USP Reference Standards, 46 Park Ave., New York 16, N.Y.

(23) To conserve space, most of the articles and some prepositions have been eliminated. Following abbreviations (most of which have been taken by permission from *Chemical Abstracts*) have been used:

Abbreviation	*Word*
Ac	CH_3CO-
addn	addition
addnl	additional
alc.	alcoholic
alk.	alkaline (*not* alkali)
alky	alkalinity
anhyd.	anhydrous
app.	apparatus
approx.	approximate(ly)
aq.	aqueous
ASTM	American Society for Testing Materials
av.	average (except as a verb)
Bé	Baumé
b.p.	boiling point

Abbreviation	*Word*	*Abbreviation*	*Word*
ca	about; approximately	ml	milliliter(s)
calc.	calculate	mm	millimeter(s)
calcd	calculated	mmg	microgram(s)
calcg	calculating	m.p.	melting point
calcn	calculation	*N*	normal (as applied to concn)
cm	centimeter(s)	NBS	National Bureau of Standards
conc.	concentrate (as a verb)	No.	number
concd	concentrated	OAc	acetate
concg	concentrating	org.	organic
concn	concentration	oz	ounce(s)
contg	containing	par.	paragraph
cryst.	crystalline (*not* crystallize)	petr. ether	petroleum ether
crystd	crystallized	pH	−log hydrogen-ion concn
crystg	crystallizing	phthln	phenolphthalein
crystn	crystallization	powd.	powdered (as an adjective)
det.	determine	ppm	parts per million
detd	determined	ppt	precipitate
detg	determining	pptd	precipitated
detn	determination	pptg	precipitating
diam.	diameter	pptn	precipitation
dil.	dilute	prep.	prepare
dild	diluted	prepd	prepared
dilg	diluting	prepg	preparing
diln	dilution	prepn	preparation
distd	distilled	qual.	qualitative
distg	distilling	quant.	quantitative
distn	distillation	resp.	respectively
elec.	electric, electrical	rpm	revolutions per minute
equiv.	equivalent	sat.	saturate
Et	ethyl	satd	saturated
evap.	evaporate	satg	saturating
evapd	evaporated	satn	saturation
evapg	evaporating	sec.	second(s)
evapn	evaporation	sep.	separate
ext.	extract	sepd	separated
extd	extracted	sepg	separating
extg	extracting	sepn	separation
extn	extraction	sol.	soluble
f.p.	freezing point	soln	solution
ft	foot, feet	std	standard
g	gram(s)	stdzd	standardized
g-s.	glass-stoppered	stdze	standardize
HOAc	acetic acid	stdzg	standardizing
hr	hour(s)	stdzn	standardization
inorg.	inorganic	sp. gr.	specific gravity
insol.	insoluble	tech.	technical
kg	kilogram	temp.	temperature
L	liter(s)	titr.	titrate
lb	pound(s)	titrg	titrating
m	meter(s)	titrn	titration
M	molar (as applied to concn, *not* molal)	USP	U. S. Pharmacopeia
max.	maximum(s)	vac.	vacuum
Me	methyl	vol.	volume (*not* volatile); also volumetric when used with flask
mg	milligram(s)	wt	weight
min.	minimum; also minute(s)	μ	micron; 0.001 mm
mixt.	mixture		

Abbreviation	*Word*
$m\mu$	millimicron; 0.000001 mm
″	inch(es)
/	per
%	per cent, percentage
>	more than, greater than, above, exceeds
<	less than, below, under

Abbreviation	*Word*
$\mathrm{\$}$	standard taper
®	trademarked

In general, principle governing use of periods after abbreviations is that period is used where final letter of abbreviation is not the same as final letter of word it represents.

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1. Agricultural Liming Materials *(1)*

1.001 Sampling—Procedure

Take sample representative of lot or shipment, avoiding disproportionate quantity of surface or of any modified or damaged zone, as follows:

(**a**) *Burnt or lump lime, in bulk.*—Collect composite sample of not <10 shovelfuls/car, with proportionate quantities from smaller lots, taking each shovelful from different part of lot or shipment. Crush immediately to pass 2″ diam. circular opening, mix thoroly and rapidly, quarter down to 5 lb sample, and place in labeled, dry, air-tight container.

(**b**) *Hydrated lime and ground burnt lime, in bags.*—Select 10 bags from different parts of each lot or shipment of 20 tons or less and 1 addnl bag for each addnl 5 tons. From each bag selected withdraw core from top to bottom with sampling tube, mix these portions thoroly and rapidly on heavy, sized paper or oilcloth, quarter down to 2 lb sample, and place in labeled, dry, air-tight container.

(**c**) *Ground limestone and ground marl, in bags.*—Proceed as in (**b**).

(**d**) *Ground limestone, ground burnt lime, ground marl, and slag, in bulk.*—With slotted sampling tube, withdraw samples to full sampler depth from 10 points in lot or shipment, and proceed as in (**b**), beginning "mix these portions . . ."

1.002 Mechanical Analysis of Ground Limestone—Procedure

Dry sample as obtained in **1.001**(**d**) in oven 16 hrs at 110°; cool to room temp.

Transfer 100 g original material to set of Nos. 10, 20, 40, 60, 80, and 100 sieves or other appropriate combination of sieves. Sift, shaking 5 min. on the Nos. 80 and 100 sieves; break lumps with soft rubber pestle if material tends to cake. Weigh material retained on each sieve and that passing No. 100 sieve and report as % total wt.

1.003 Preparation of Sample—Procedure

Grind sample in porcelain mortar or porcelain ball mill to pass No. 60 sieve, mix thoroly, and store in air-tight container.

Neutralizing Value—Official

(Uncorrected for sulfide content)

1.004 REAGENTS

(**a**) *Sodium hydroxide std soln.*—0.25*N*. Prep. free from carbonates and store in Pyrex bottle provided with siphon tube and with guard tubes contg soda-lime, or other suitable device, to prevent absorption of CO_2 from air. Stdze as in **42.033** or **42.034**.

(**b**) *Hydrochloric acid std soln.*—0.5*N*. Stdze against (**a**), using phthln.

1.005 *Indicator Titration Method*

Place 0.5 g burnt or hydrated lime (1 g ground limestone or ground marl), prepd as in **1.003**, in 250 ml erlenmeyer; add 50 ml of the HCl and boil gently 5 min. Cool, and titr. excess acid with the NaOH, using phthln. For burnt and hydrated lime report as % CaO; for limestone and marl report as % $CaCO_3$ equivalence. % $CaCO_3$ equivalence of sample = 2.5 × (ml HCl − ml NaOH/2).

1.006 *Potentiometric Titration Method*

(Applicable to liming materials, other than silicate slags, contg large amounts of ferrous Fe or coloring matter)

Proceed as in **1.005** thru "Cool . . ." Transfer to 250 ml beaker and insert glass and calomel electrodes of pH meter, buret contg 0.25*N* NaOH, and mechanical stirrer. Stir at moderate speed to avoid splash. Deliver NaOH rapidly to pH 5, then dropwise until soln attains pH 7 and remains constant 1 min. while stirring. (If end

point is passed, add, from 1 ml Mohr pipet, just enough 0.5N HCl to bring pH to <7, and back-titr. slowly to pH 7.) Add ml of excess acid, if used, to initial 50 ml in calcg. Report as % $CaCO_3$ equivalence as in **1.005**.

1.007 *Approximate Proportions of Calcium and Magnesium in Magnesic Limestone*

Slightly acidify titrd soln, **1.005** or **1.006**, transfer to 250 ml vol. flask, and dil. to vol. Det. Ca in 50 ml aliquot as in **22.057**, beginning "dil. to ca 100 ml . . . " Subtract its $CaCO_3$ equivalence from total $CaCO_3$ equivalence, **1.005** or **1.006**, and assign difference as $CaCO_3$ equivalence of the Mg content of the dolomite.

Caustic Value (*2*)—Official

1.008 APPARATUS (FIGURE 1)

Use 500 ml Pyrex erlenmeyer, *A*, and fritted glass filter (Corning 39535, 30F), *F*. Connect filter to siphon tube *B* with thick-wall rubber tubing. Use receiving flasks *M* and *N* calibrated to *deliver* 50 and 100 ml, resp. *S* is suction flask.

1.009 DETERMINATION

Transfer portion of sample, **1.003**, to weighing bottle and det. wt bottle and contents in atmosphere of min. moisture and CO_2 content. With polished, narrow-point spatula calibrated to hold ca 1.5 g, withdraw charge to be used and det. exact wt by difference. Insert charge directly into dry flask, *A*, fitted with tight rubber stopper.

Prep. *sucrose soln immediately before use* by placing 25 g granulated sucrose in measuring flask calibrated to *deliver* 500 ml. Dissolve sucrose with cold *CO_2-free H_2O* and dil. to mark. Holding both erlenmeyer contg charge and flask contg sucrose soln in slightly inclined position, insert neck of sucrose soln flask short distance into erlenmeyer, and carefully transfer sucrose soln with synchronized rotary motion of both flasks to prevent granulation of lime. Stopper erlenmeyer securely, agitate, and add, if desired, quantity of clean dry beads. Completely dissolve uncoated caustic lime by six 1 min. agitations at 2 or 3 min. intervals. Crush any solid particles not disintegrated by inverting flask to trap particles between stopper and neck of flask and carefully twisting stopper. Let stand 15 min. and filter as follows:

Connect filter cone *F* with siphon *B* and close stopcock *D*. Connect receiving flasks, apply suction, and quickly connect erlenmeyer *A* contg lime soln with stopper *E*. Open stopcock *C* and filter 25–50 ml soln. Close *C* and open *D* to release suction. Remove *M* and replace with similar dry flask. Close *D*, open *C*, and continue filtration until both *M* and *N* are filled at least to marks. To disconnect system, close stopcock *C*, and gently

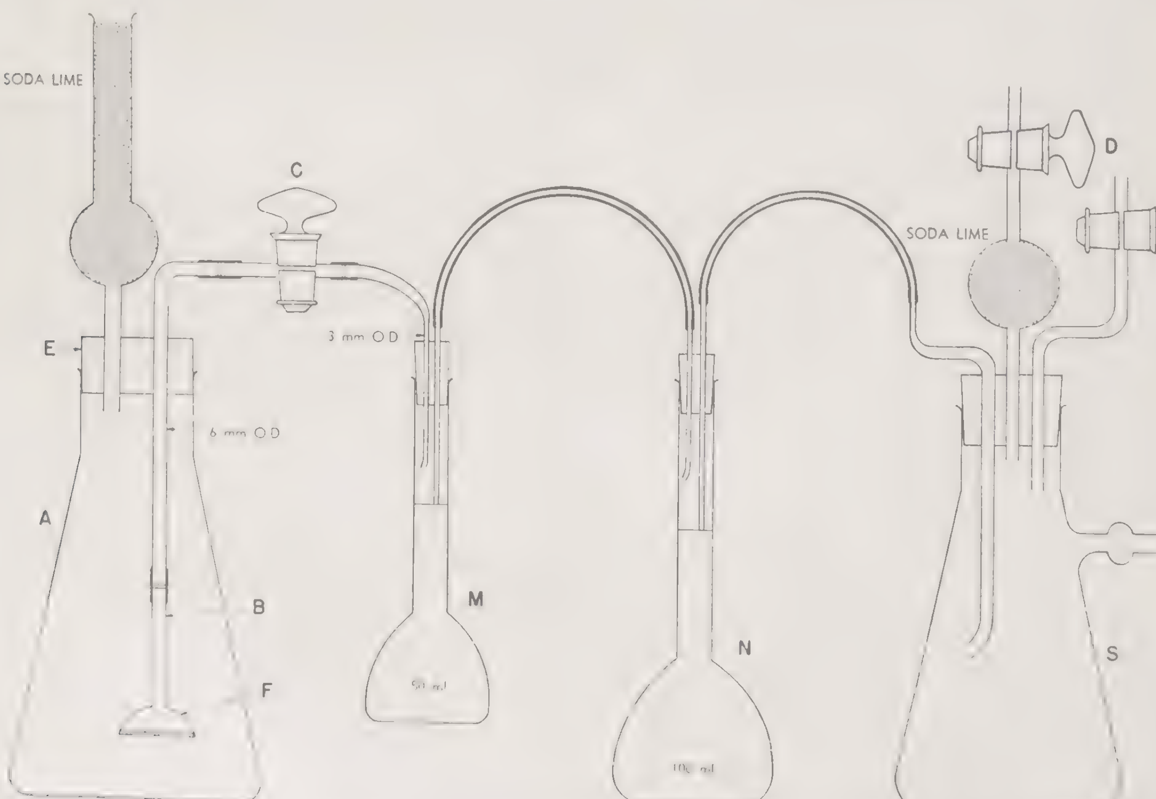

FIG. 1.—APPARATUS FOR AUTOMATIC FILTRATION AND MEASUREMENT OF LIME SOLUTIONS

press down outlet of flask *M* and then outlet of flask *N*, to remove any excess of liquid above marks. Let intermediate connection empty, open stopcock *D*, and remove *M* and *N*. Titr. first 50 ml, or pilot aliquot, of filtered soln with 0.5*N* HCl, using phthln. Add to covered 200 ml beaker twice the vol. 0.5*N* acid required for this titrn; add second, or 100 ml aliquot, of filtered soln to this acid and phthln; and complete titrn.

Calc. caustic value of sample by formula: $X = 7A/W$, in which $X = \%$ active CaO; $A =$ ml 0.5*N* acid used/100 ml lime soln; and $W =$ wt sample.

1.010 Carbon Dioxide—First Action

Proceed as in **2.080–2.081**, using 3 g burnt or hydrated lime (0.5–1.0 g limestone or marl) prepd as in **1.003**. Report as % $CaCO_3$.

CALCIUM SILICATE SLAGS

1.011 Neutralizing Value (*3*)—First Action

(Uncorrected for sulfide content)

(**a**) *Blast furnace slag.*—Transfer 0.5 g sample, ground to pass No. 80 sieve, to 250 ml erlenmeyer. Wash down with small portions of H_2O and add 35 ml 0.5*N* HCl while swirling. Heat to gentle boil over burner, *agitating suspension continuously* until bulk of sample dissolves. Boil 5 min. and cool to room temp.; then dil. with CO_2-free H_2O to ca 150 ml and add 1 ml *30% H_2O_2* and 5 drops bromocresol green **2.093(e)**. Back-titr. with 0.5*N* NaOH, adding first 15 ml rapidly and titrg dropwise thereafter, vigorously agitating contents of stoppered flask after each addn, until indicator tint matches or slightly exceeds that of pH 5.2 phthalate buffer soln, **13.024**, of like vol. and indicator concn after 2–3 sec. agitation.

(**b**) *Rock phosphate reduction furnace slag.*—Transfer 0.5 g sample to 250 ml beaker. Wash down with small portions of H_2O and add, stirring continuously, 50 ml HOAc (1+4). Heat to boiling and boil 5 min., stirring frequently. Evap. to dryness on steam bath. Add 20 ml of the HOAc, dil. to 150 ml, and heat to boiling; add NH_4OH (1+1) to distinct yellow of Me red, **1.012(e)**. Digest ca 10 min. on hot plate. Filter by gravity thru 9 cm paper, catching filtrate in 100×50 mm lipped Pyrex crystg dish; wash beaker 3 times and filter 5 addnl times with neutral 0.5*N* NH_4OAc. Evap. filtrate on hot plate. Adjust heat so bubbles breaking thru viscous surface film are released gently to avoid spattering. (To expedite dehydration repeat treatments with 25 ml hot H_2O and evapn 2 or 3 times.) Continue heating residue on hot plate until no HOAc odor remains. Heat addnl 10 min. at full heat of hot plate and then ignite 10 min. in muffle at 550°. Cool, wet residue with 15 ml H_2O, place watch glass over dish, and add 25 ml 0.5*N* HCl thru lip of dish. Heat 5 min. over burner at gentle simmer. Rinse watch glass, filter suspended matter on 9 cm paper, catching filtrate in 250 ml erlenmeyer, and wash dish and filter 3 times with hot H_2O. Titr. excess acid with 0.5*N* NaOH to distinct yellow of Me red. Net acid used ×5 = neutralizing value of slag in terms of % $CaCO_3$ equivalence.

Sulfide Sulfur (*4*)—First Action

1.012 REAGENTS

(**a**) *Zinc dust.*—Low in Pb.

(**b**) *Absorbent.*—Dissolve 20 g $CdSO_4.2\frac{2}{3}H_2O$ in H_2O and dil. to 1 L. Adjust to pH 5.6 potentiometrically or colorimetrically; if colorimetrically, match sep. 50 ml aliquot to buffer of same pH, **13.024**.

(**c**) *Standard alkali.*—Prep. 0.1*N* NaOH from CO_2-free NaOH soln and CO_2-free H_2O. Stdze as in **42.033** or **42.034**.

(**d**) *Standard acid.*—0.1*N* HCl. Stdze against the std alkali, (**c**), using Me red.

(**e**) *Methyl red indicator.*—Dissolve 0.2 g Me red in 100 ml alcohol.

1.013 APPARATUS

Fit 250 ml erlenmeyer with 2 hole No. 5.5 stopper. Insert thru stopper 60 ml separator with stem drawn out to 2 mm and bent upward at tip, adjusting separator so stem is ¼″ from bottom of flask. Also insert thru stopper 6 mm glass outlet tube. Connect with amber rubber tubing to inlet of 25×50 mm tube half filled with H_2O and heated to near boiling before and during detn. Connect in series 2 addnl tubes of same size, each contg 25 ml absorbent soln and held in 600 ml beaker filled with cold H_2O.

1.014 DETERMINATION

Charge absorbent tubes with the $CdSO_4$ soln and heat H_2O tube to gentle boiling. Weigh 1 g −80-mesh slag into evolution flask, add 1 g of the Zn dust, and wash down sides with 5–10 ml H_2O; mix contents with flat-end rod and connect flask to app. Add 50 ml HCl (1+4) to separator and let acid flow into reaction flask while swirling contents. If necessary, apply pressure to transfer acid and close stopcock while a little of the acid is still above it. Heat until boiling starts; then regulate to maintain active but not too vigorous boiling for 10 min. Swirl flask frequently after adding acid and for first 5 min. of boiling. To disconnect, hold inlet in first absorbent tube firmly with one hand and quickly pull off rubber tubing with other hand without pinching.

Filter CdS suspension by gravity on 9 cm paper into 250 ml erlenmeyer and wash with H_2O to vol. of 100 ml. Add 4 drops of the Me red soln and agitate vigorously while titrg slowly with 0.1*N* NaOH to exact tint of *reference soln* (50 ml of the $CdSO_4$ soln dild to 100 ml, with identical concn of indicator, in 250 ml erlenmeyer). If end point is passed so that $Cd(OH)_2$ ppts, add 1–2 ml 0.1*N* HCl, let stand until ppt disappears, and complete titrn dropwise, agitating vigorously. Net ml 0.1*N* NaOH/2 = % $CaCO_3$ equivalence of sulfide *S* in sample. Ml 0.1*N* NaOH × 0.0016 = g sulfide *S*/detn; g sulfide *S* × 100 = % sulfide *S*.

ELEMENTARY ANALYSIS

1.015 Sodium Carbonate Fusion (*5*)—First Action

In 30 ml Pt crucible, mix 2 g sample with 10 g Na_2CO_3. Cover crucible and heat at low redness until fusion begins; increase heat to clear, quiet fusion; then use full heat of Meker burner 20 min., with flame oblique. Cool melt, place in 250 ml porcelain evapg dish, add 100 ml H_2O, and digest to disintegration on H_2O bath. Cover dish, add 50 ml HCl, digest 15 min., and wash cover. Evap. to dryness and bake 2 hr at 110° (or substitute covered beaker for dish and 15 ml $HClO_4$ for HCl, and evap. to fumes on hot plate, thereby obviating baking). (*See* section on $HClO_4$ in *Definitions of Terms and Explanatory Notes.*)

1.016 Silica—First Action

Take up residue from **1.015** in HCl (1+9) and filter. Wash with hot H_2O contg 5 ml HCl/L. Collect filtrate and washings in casserole, evap. soln, dehydrate on steam bath until SiO_2 appears cryst., and then heat 1 hr at 110°. Add 5 ml HCl and 100 ml hot H_2O, mix thoroly, filter, and wash with hot H_2O contg 5 ml HCl/L. Add residue to main portion SiO_2 obtained from first filtration. Dil. combined filtrate and washings to 500 ml.

Place the two SiO_2 residues with filters in Pt crucible. Moisten with satd NH_4NO_3 soln. Ignite at low heat to burn off paper and then with intense flame, preferably blast lamp, to constant wt; cool in desiccator and weigh. Report as % SiO_2.

1.017 Oxides of Iron, Aluminum, Phosphorus, and Titanium—First Action

To 250 ml aliquot filtrate from **1.016,** add 10 ml HCl and few drops Me red indicator; heat to gentle boiling and add NH_4OH (1+1) until ppt forms and indicator just changes to distinct yellow. Boil not longer than 2 min. and filter rapidly. Wash ppt 6–8 times with hot *2%* NH_4NO_3 soln. Return ppt and filter to original beaker, add 10 ml HCl, and macerate filter with policeman. Dil. with H_2O, heat to dissolve ppt, dil. to ca 200 ml, and reppt as above. Wash thoroly with the hot NH_4NO_3 soln until Cl-free. Combine first and second filtrates and save for Ca and Mg detns.

Place ppt in Pt crucible; dry; ignite gently to oxidize C; then heat to bright red ca 10 min., cool in desiccator, and weigh in covered crucible as Fe_2O_3, Al_2O_3, P_2O_5, and TiO_2.

1.018 Calcium—First Action

Conc. combined filtrates and washings from **1.017** to ca 50 ml; make slightly alk. with NH_4OH (1+1); while still hot, add satd $(NH_4)_2C_2O_4$ soln dropwise as long as any ppt forms, and then excess sufficient to convert Mg salts also to oxalate. Heat to boiling, let stand 3 hr or longer, decant clear soln thru filter, pour 15–20 ml hot H_2O on ppt, and again decant clear soln thru filter. Dissolve any ppt remaining on filter by washing with hot HCl (1+9) into original beaker, wash 6 times with hot H_2O, and then reppt boiling hot, by adding NH_4OH and a little satd $(NH_4)_2C_2O_4$ soln. Let stand as before, filter thru same filter, and wash with hot H_2O until Cl-free. Reserve filtrates and washings from both pptns for detn of Mg, **1.020.**

Complete detn by one of following methods and report as % CaO:

(**a**) Ignite ppt in crucible either over S-free blast or in elec. furnace at 950° to constant wt, cool in desiccator, and weigh as CaO.

(**b**) Incinerate filter over low flame, mix ignited ppt with finely pulverized and dried mixt. of equal parts of $(NH_4)_2SO_4$ *and* NH_4Cl, and drive off excess sulfate by careful heating of upper portion of crucible. Complete ignition, cool in desiccator, and weigh the $CaSO_4$ (*6*).

(**c**) Perforate apex of cone; wash CaC_2O_4 ppt into beaker used for pptn; then wash filter with hot H_2SO_4 (1+4) and titr. at 85–90° with 0.1*N* $KMnO_4$.

1.019 Magnesium (*7*)—First Action

REAGENT

Phosphate soln.—Dissolve 100 g $(NH_4)_2HPO_4$ in hot H_2O, dil. to 1 L, and add 5 ml $CHCl_3$.

1.020 DETERMINATION

To combined filtrates and washings, **1.018,** add 2 ml 1*M* citric acid, 100 ml NH_4OH, and 50 ml alcohol. Then add with constant stirring 25 ml of the phosphate soln and let stand 12–24 hr. Filter,

wash twice with NH_4OH (1+9), and dissolve ppt in HNO_3 (1+4), washing soln into original beaker to vol. of 100–150 ml. Add 1/10 vol. NH_4OH and 2 drops of the phosphate soln. Stir vigorously and let stand 3 hr or longer. Filter thru gooch, wash with NH_4OH (1+9), moisten filter with *satd ammoniacal soln of NH_4NO_3*, ignite, and weigh as $Mg_2P_2O_7$. Report as % MgO. Correct wt $Mg_2P_2O_7$ for co-pptd $Mn_2P_2O_7$ as in **31.036**.

SELECTED REFERENCES

(*1*) J. Assoc. Offic. Agr. Chemists **7,** 252(1924).
(*2*) Ind. Eng. Chem. **20,** 312(1928); J. Assoc. Offic. Agr. Chemists **11,** 153(1928); **14,** 283(1931).
(*3*) J. Assoc. Offic. Agr. Chemists **27,** 74, 532 (1944); **28,** 310(1945); **31,** 71(1948).
(*4*) Ibid. **31,** 715(1948).
(*5*) U.S. Geol. Survey Bull. **700,** p. 94.
(*6*) Ind. Eng. Chem. **9,** 1114(1917).
(*7*) Washington, "Chemical Analysis of Rocks," 3rd ed., 1919, p. 181.

2. Fertilizers

Sampling

2.001 *Solid Fertilizers (1)—Official*

Use slotted single or double tube, or slotted tube and rod, with solid cone tip at one end. Take sample as follows: Lay bag horizontally and remove core diagonally from end to end. From lots of 10 bags or more, take core from each of 10 bags. When necessary to sample lots of <10 bags, take 10 cores but at least 1 core from each bag present. For bulk fertilizers, draw at least 10 cores from different regions. Bulk shipments may be sampled at time of loading or unloading by passing container thru entire stream of material as it drops from transfer belt or chute. For small packages (10 pounds or less) take 1 entire package as sample. Reduce composite to quantity required, preferably by riffling, or by mixing thoroly on clean oilcloth or paper and quartering. Place sample in airtight container.

2.002 *Liquid Fertilizers (In Absence of Free Ammonia) (2)—Official*

Flush delivery line and faucet on storage tank and collect enough sample in glass or polyethylene container. Alternatively, lower sample container into material thru port in top of tank and let fill. Seal container tightly to prevent evapn.

Ammoniacal Solutions (2)—Official

2.003 APPARATUS

(a) *Container.*—Polyethylene reagent-form bottle with buttress-type cap, 1 qt. capacity.

(b) *Sample flow control apparatus.*—Construct from following fittings: $1\frac{1}{2}\times\frac{1}{4}''$ reducing bushing; $\frac{1}{4}''$ tee; $\frac{1}{4}''$ nipple 12–18″ long (length not critical); two $\frac{1}{4}''$ stainless steel, blunt-nose needle valves with hose connections (Hoke No. 328). All fittings except valves can be either Al or stainless steel. (*See* Fig. 2.)

Attach valves directly to tee which is then attached to reducing bushing thru nipple. To both valves attach $\frac{1}{4}''$ i.d. Tygon tubing (Hoke No. 314A hose connection); 12″ length to sample valve and sufficient length to vent valve to reach disposal area or container. To free end of sample tubing attach 3″ length of $\frac{1}{4}''$ glass or stainless steel tubing inserted thru No. 4 rubber stopper. To exit end of metal tube attach addnl 6″ length of Tygon tubing. Make certain all connections are tight. App. can be attached directly to tank cars, but requires addnl coupling, which varies with installation, to attach to storage tanks. $1\frac{1}{2}''$ "quick coupler" (O P W Corp., Cincinnati 25, Ohio, or Ever-Tite Coupling Co., New York 19, N.Y.) suffices in most cases.

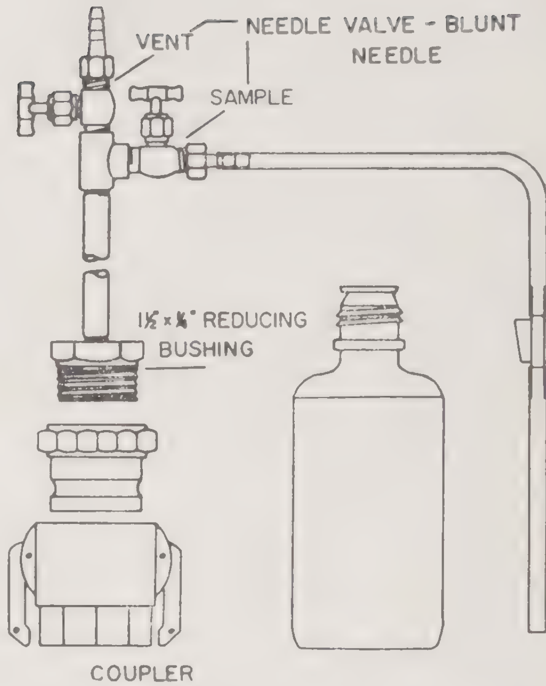

FIG. 2.—SAMPLING APPARATUS FOR AMMONIACAL SOLUTIONS, INCLUDING "QUICK COUPLER" FOR ATTACHING TO STORAGE TANKS

2.004 SAMPLING

Prep. sample bottle in laboratory by adding ca 500 ml H_2O, replacing cap, and weighing accurately (±0.1 g). Attach sampling app. to car or tank and, with sample valve closed, flush line thru vent valve. Partially collapse sample bottle, insert sample tube with stopper, and seat tightly. With sample tube dipping below surface of H_2O in bottle, throttle vent valve to maintain small flow of soln and partially open sample valve, collecting ca 100 ml sample. (Bottle should not expand to full size during this time.) Close sample valve, remove sample tube, partially collapse bottle, and cap tightly. Reweigh (±0.1 g) and calc. wt sample. Cool to 20°, transfer to 1 or 2 L vol. flask, dil. to vol. with H_2O, mix thoroly, and take aliquots for analysis.

Anhydrous Ammonia (2)—Official

(Use extreme care in handling anhyd. NH_3. Suitable gas mask and rubber gloves are required).

2.005 SAMPLING

Use sample tube of thermal shock-resistant glass calibrated to contain 100 ml and graduated in 0.05 ml subdivisions up to 0.5 ml. (DuPont special oil centrifuge tube or ASTM long-form oil tube is satisfactory.) Flush line and fill tube to 100 ml mark with sample in such manner that condensing moisture will not enter sample tube. (Skirt attached to end of sample line will drain moisture away.)

2.006 DETERMINATION OF WATER AND NITROGEN

Close sample tube immediately with tight-fitting rubber stopper into which is inserted tight-fitting piece of 0.25″ i.d. glass tubing 2–3″ long, bent at its exit from outer end of stopper to let gases escape but to exclude entrance of moisture or moisture-laden air. Place in H_2O bath at ca air temp. and let NH_3 evap. When temp. of sample tube is ca that of bath, remove tube, wipe outer surface, and det. vol. of residue.

% H_2O in sample = ml residue $\times C$, where $C = 0.74$, 0.70, or 0.66 for pressures in original containers of 100, 150, or 200 lbs/sq. in., resp.

$$\%\ N = (100 - \%\ H_2O) \times 0.8224.$$

2.007 Preparation of Sample (*3*)—Official

Reduce gross sample to quantity sufficient for analysis or grind not <0.5 lb of reduced sample without previous sieving. For fertilizer materials and moist fertilizer mixts, grind to pass sieve with 1 mm circular openings, or No. 20 std sieve; for dry mixts that tend to segregate, grind to pass No. 40 std sieve. Grind as rapidly as possible to avoid loss or gain of moisture during operation. Mix thoroly and store in tightly stoppered bottles.

2.008 Mechanical Analysis of Bone, Tankage, and Basic Slag—Official

Transfer 100 g original bone or tankage or 10 g basic slag to sieve with circular openings 0.5 mm diam., and sift. Break lumps with soft rubber pestle if material tends to cake. Weigh coarse portion remaining on sieve. Det. fine portion by difference.

Mechanical Analysis of Phosphate Rock (*4*)—Official

2.009 APPARATUS

(a) *Water pressure control.—See* Fig. 3. Connect valve, *A*, std pressure gauge, *B*, and aerator, *C*, with ⅜″ diam. pipe.

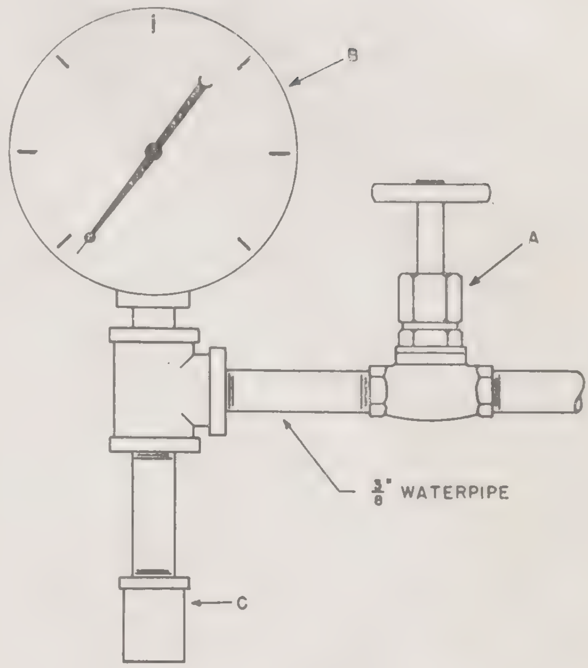

FIG. 3.—APPARATUS FOR CONTROL OF WATER PRESSURE

(b) *Sieves.*—Nos. 100 and 200, bronze or stainless steel cloth, checked against certified sieves. Sieves 8″ diam. and 2″ in depth to sieve cloth are recommended for both wet and dry sieving, but other sizes may be used if detd to be suitable under conditions of method. (Other sieves in U. S. series may be used, with precaution to insure complete sepn of sample into desired fractions.)

(c) *Sieve shaker.*—Ro-Tap, Syntron, or other suitable machine.

2.010 REAGENT

Dispersing agent.—Dissolve 36 g Na hexametaphosphate and 8 g Na_2CO_3 in H_2O and dil. to 1 L.

2.011 DETERMINATION

(a) *Ground phosphate rock.*—Place 100 g sample on No. 200 sieve and wash with moderate stream of tap H_2O at max. gauge pressure of 4 lb until H_2O passing sieve is clear, with care to avoid loss of sample by splashing. Dry material remaining on sieve at 105° and transfer to No. 100 sieve in series with No. 200 sieve of same diam. and depth. Shake 8 min. in mechanical shaker. Det. % sample passing No. 100 sieve by subtracting wt of material retained on that sieve from 100. Det. % sample passing No. 200 sieve by subtracting sum of wts of material retained on that sieve and on No. 100 sieve from 100.

(b) *Soft phosphate with colloidal clay.*—Add

100 g sample to rapidly stirred soln of 50 ml dispersing agent and 450 ml tap H_2O, with care to avoid contact of unwetted material with shaft of stirrer and side of beaker. Stir 5 min. after addn of sample is completed. Transfer slurry to No. 200 sieve and proceed as in (a).

2.012 Total Water—Official

(Not applicable to samples that yield volatile substances other than H_2O at drying temp.)

Heat 2 g sample, **2.007,** 5 hr in oven at 99–101°. In case of $NaNO_3$, $(NH_4)_2SO_4$, and K salts, heat to constant wt at 129–131°. Report % loss in wt as H_2O at temp. used.

Free Water (*5*)

Air-flow Method—Official

2.013 APPARATUS

(**a**) *Manifold.*—Six-crucible manifold, Fig. 4, requires following pipe fittings: 5 tees, $\frac{1}{2}\times\frac{1}{2}\times\frac{1}{4}''$; 2 ells, $\frac{1}{2}\times\frac{1}{4}''$; 2 ells, $\frac{1}{2}''$; 8 close nipples, $\frac{1}{2}''$; 7 nipples, $\frac{1}{4}''$ diam., $1\frac{1}{2}''$ long (preferably tapered at one end to remove threads).

Provide crucible seats by placing 1-hole No. 6 rubber stoppers over vertical $1\frac{1}{2}''$ nipples. (Nipples should extend ca $\frac{1}{8}''$ above stoppers to facilitate centering of crucibles over openings.) Grind lower edge of crucible and upper surface of stopper smooth to insure proper seal, since all connections must be air-tight when vac. is on.

(**b**) *Crucibles.*—Pyrex glass, ca $1\frac{3}{4}''$ tall, $1\frac{3}{16}''$ diam. with 20 mm fine porosity fritted glass plate. Select set of crucibles matched for porosity by taking those that pass given vol. of air under same conditions of temp. and pressure in ca same time.

(**c**) *Vacuum gauge.*—Std instrument for insertion in vac. line between source of vac. and manifold.

(**d**) *Constant temperature oven.*—Std laboratory oven, preferably vented so that incoming air passes directly over heating coils.

2.014 DETERMINATION

Weigh 2 g prepd sample, **2.007,** in tared, fritted glass crucible, **2.013**(**b**). (Weigh extremely hygroscopic or damp materials by difference in covered crucibles.) Place crucible on manifold in oven at 60°. Aspirate under 15″ vac. 2 hr. Cool in desiccator 30 min. and reweigh. Report % loss in wt as free H_2O.

2.015 *Vacuum-Desiccation Method—Official*

Place 2 g prepd sample, **2.007,** in tared, low-form weighing dish. (Weigh extremely hygroscopic or damp materials by difference in covered dishes; if dishes $1\frac{1}{2}$–2″ diam. are used, sample may be as large as 4 g.) Dry sample at 25–30° in vac. desiccator over *anhyd.* $Mg(ClO_4)_2$, or equiv. desiccant, under not <20″ of vac. 16–18 hr. (Most fertilizers give up their free H_2O within 3–8 hr, but overnight drying of 16–18 hr insures best results on damp, porous materials.) Reweigh, and report % loss in wt as free H_2O.

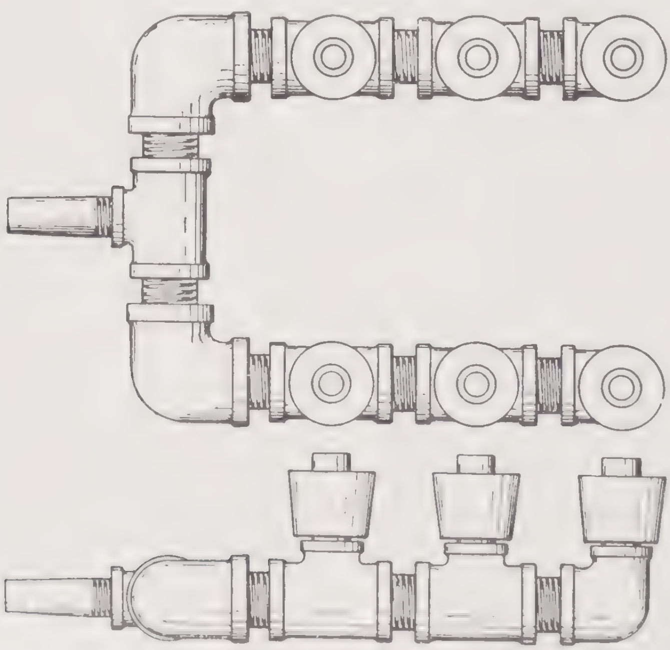

FIG. 4.—VIEWS OF MANIFOLD ASSEMBLED WITH PIPE FITTINGS

2.016 Acid-Insoluble Ash (6)—Official

Transfer 2 g sample to 400 ml beaker. Add 100 ml HCl (1+4), cover with watch glass, and immerse 30 min. in steam or hot H_2O bath (98–100°), keeping liquid level in beaker below that of H_2O in bath. Stir at 10 min. intervals, and after 30 min. remove from bath and filter thru 11 or 12.5 cm medium paper, transferring insol. residue to filter with stream of H_2O. Fold paper contg residue, place in porcelain crucible, and ignite in muffle furnace 1 hr at 800°. Cool, transfer contents of crucible to original beaker with 50 ml HCl (1+4), cover, and again immerse in steam or hot H_2O bath 30 min., stirring occasionally. After 30 min., remove from bath and filter thru tared gooch contg acid-washed asbestos mat on filter paper disk. Wash insol. residue several times with H_2O, dry crucible 1 hr at 125°, cool in desiccator, and weigh. Calc. net increase in wt crucible to % acid-insol. ash.

PHOSPHORUS

Total Phosphorus

Gravimetric Method—Official

2.017 REAGENTS

(**a**) *Molybdate soln.*—Dissolve 100 g MoO_3 in mixt. of 144 ml NH_4OH and 271 ml H_2O. Cool, and pour soln slowly, stirring constantly, into cool mixt. of 489 ml HNO_3 and 1148 ml H_2O. Keep final mixt. in warm place several days or until portion heated to 40° deposits no yellow ppt. Decant soln from any sediment and keep in g-s. vessels.

(**b**) *Ammonium nitrate soln.*—Dissolve 100 g P-free NH_4NO_3 in H_2O and dil. to 1 L.

(**c**) *Magnesia mixture.*—(1) Dissolve 55 g crystd $MgCl_2.6H_2O$ in H_2O, add 140 g NH_4Cl and 130.5 ml NH_4OH, and dil. to 1 L. Or, (2) dissolve 55 g crystd $MgCl_2.6H_2O$ in H_2O, add 140 g NH_4Cl, dil. to 870 ml, and add NH_4OH to each required portion of soln just before using, at rate of 15 ml /100 ml soln.

(**d**) *Ammonium hydroxide soln for washing.*—(1+9). Should contain not <2.5% NH_3 by wt.

(**e**) *Magnesium nitrate soln.*—Dissolve 950 g P-free $Mg(NO_3)_2.6H_2O$ in H_2O and dil. to 1 L.

2.018 PREPARATION OF SOLUTION

Treat 2 g sample by (**a**), (**b**), (**c**), (**d**), or (**e**). Cool soln, transfer to 200 ml vol. flask, dil. to vol., mix, and filter thru dry filter.

(**a**) (Suitable for materials contg small quantity of org. matter.) Dissolve in 30 ml HNO_3 and 3-5 ml HCl, and boil until org. matter is destroyed.

(**b**) (Suitable for fertilizers contg much Fe or Al phosphate and for basic slag.) Dissolve in 15–30 ml HCl and 3–10 ml HNO_3.

(**c**) (Suitable for org. material like cottonseed meal alone or in mixts.) Evap. with 5 ml of the $Mg(NO_3)_2$ soln, ignite, and dissolve in HCl.

(**d**) (Generally applicable to materials or mixts contg large quantities of org. matter. With cottonseed meal and similar materials it is best to add first ca 5 ml HNO_3 and then the H_2SO_4.) Boil with 20–30 ml H_2SO_4 in 200 ml flask, adding 2–4 g $NaNO_3$ or KNO_3 at beginning of digestion and small quantity after soln is nearly colorless, or adding the nitrate in small portions from time to time. When soln is colorless, cool, add 150 ml H_2O, and boil few min. Before adding the $NaNO_3$ or KNO_3, let mixt. digest, at gentle heat if necessary, until violence of reaction is over.

(**e**) (Suitable for all fertilizers.) (*See* section on $HClO_4$ in *Definitions of Terms and Explanatory Notes.*) Boil gently 30–45 min. with 20–30 ml HNO_3 in suitable flask (preferably Kjeldahl for samples contg large quantities of org. matter) to oxidize all easily oxidizable matter. Cool and add 10–20 ml 70–72% $HClO_4$. Boil very gently until soln is colorless or nearly so and dense white fumes appear in flask. Do not boil to dryness at any time (Danger!). (With samples contg large quantities of org. matter, temp. should be raised to fuming point, ca 170°, over period of at least 1 hr.) Cool slightly, add 50 ml H_2O, and boil few min.

2.019 DETERMINATION

Pipet aliquot of prepd soln corresponding to 0.25 g, 0.50 g, or 1 g sample, into 250 ml beaker; add NH_4OH in slight excess and barely dissolve ppt formed with few drops HNO_3, stirring vigorously. If HCl or H_2SO_4 has been used as solvent, add ca 15 g cryst. NH_4NO_3 or soln contg that quantity. To hot soln add 70 ml of the molybdate soln for every 100 mg P_2O_5 present. Digest 1 hr at ca 65° and test for complete pptn of P_2O_5 by adding more molybdate soln to clear supernatant. Filter, and wash with cold H_2O or preferably with the NH_4NO_3 soln. Dissolve ppt on filter with NH_4OH (1+1) and hot H_2O, and wash into beaker to vol. not >100 ml. Neutralize with HCl, using litmus paper or *bromothymol blue* as indicator; cool, and from buret add slowly (ca 1 drop/sec.), stirring vigorously, 15 ml of the magnesia mixt./100 mg P_2O_5 present. After 15 min. add 12 ml NH_4OH and let stand until supernatant is clear (usually 2 hr); filter, wash ppt with NH_4OH (1+9) until washings are practically Cl-free, dry, burn at low heat, and ignite to constant wt, preferably in elec. furnace at 950–1000°; cool in desiccator, and weigh as $Mg_2P_2O_7$. Report as % P_2O_5.

With basic slag, dehydrate 20 ml aliquot of prepd soln by evapg to dryness on steam or hot H_2O bath. Treat with 5 ml HCl and 25 ml hot H_2O, digest in order to complete soln, and filter off SiO_2. Proceed as above. Before pptg with magnesia mixt. add 5 ml *5% NaOAc soln.*

Volumetric Method (7)—Official

2.020 REAGENTS

(a) *Molybdate soln.*—To 100 ml molybdate soln, **2.017(a)**, add 5 ml HNO_3. Filter immediately before using.

(b) *Sodium or potassium hydroxide std soln.*—Dil. 324.03 ml 1*N* alkali, carbonate-free, **42.030–42.034,** to 1 L. (100 ml of this soln should neutralize 32.40 ml 1*N* acid; 1 ml = 1 mg or 1% P_2O_5 on basis of 0.1 g sample.) For basic slag, stdze against std phosphate material of ca same composition as sample being examined. (Since burets in constant use may become so corroded as to increase their capacity, test them at least annually.)

(c) *Std acid soln.*—Prep. soln of HCl or of HNO_3, corresponding to concn of (b) or to ½ this concn, and stdze by titrn against that soln, using phthln.

2.021 PREPARATION OF SOLUTION

(a) Treat 2 g sample as in **2.018(a)**, **(b)**, **(c)**, **(d)**, or **(e)**, preferably (a) when these acids are suitable solvent.

(b) Proceed as in **2.018(a)**, **(b)**, **(c)**, or **(e)**, preferably (a) when these acids are suitable solvent. Add 25 ml *10% $BaCl_2$ soln* to hot digestate, boil ca 2 min., cool, dil. to 200 ml, mix, filter thru dry filter, and continue as in **2.022(b)**.

2.022 DETERMINATION

(a) Prep. sample soln as in **2.021(a)**. Pipet, into beaker or flask, aliquot corresponding to 0.4 g sample for P_2O_5 content of sample <5%; 0.2 g for 5–20%; 0.1 g for >20%. Add 5–10 ml HNO_3, depending on method of soln (or equiv. in NH_4NO_3); then add NH_4OH until ppt that forms dissolves only slowly on vigorous stirring, dil. to 75–100 ml, and adjust to 25–30°. If sample does not give ppt with NH_4OH as test of neutralization, make soln slightly alk. to litmus paper with NH_4OH and then slightly acid with HNO_3 (1+3). Add 20–25 ml of the molybdate soln for P_2O_5 content <5%; 30–35 ml for 5–20%; and enough molybdate soln to insure complete pptn for >20%. Place soln in shaking or stirring app. and agitate 30 min. at room temp.; decant *at once* thru filter and wash ppt twice by decanting with 25–30 ml portions H_2O, agitating thoroly and allowing to settle. Transfer ppt to filter and wash with cold H_2O until filtrate from 2 fillings of filter yields pink color on adding phthln and 1 drop of the std alkali. Transfer ppt and filter to beaker or pptg vessel, dissolve ppt in small excess of the std alkali, add few drops of the phthln, and titr. with the std acid. Report as % P_2O_5.

(b) Prep. soln as in **2.021(b)**. Proceed as in **2.022(a)** to diln to 75–100 ml. Heat in H_2O bath to 45–50°, add the molybdate soln at rate of 75 ml /100 mg P_2O_5 present, and let mixt. remain in bath 30 min., stirring occasionally. Decant *at once* thru filter, wash, and titr. as in **2.022(a)**.

(c) *Not applicable to superphosphates and other fertilizers contg sulfate or to solns prepd with aid of sulfuric acid.*—Prep. soln of sample as in **2.021(a)**. Proceed as in **(b)**.

Photometric Method (8)—Official

(Not applicable to materials yielding colored solns or solns contg ions other than orthophosphate which form colored complexes with molybdovanadate. Not recommended for basic slag.)

2.023 APPARATUS

Photometer.—Beckman Model DU spectrophotometer with stray light filter and matched 1 cm absorption cells. With other photometers analyst must det. suitability for use and conditions for satisfactory performance. Means for dispelling heat from light source is desirable.

2.024 REAGENTS

(a) *Molybdovanadate reagent.*—Dissolve 40 g NH_4 molybdate tetrahydrate in 400 ml hot H_2O and cool. Dissolve 2 g NH_4 metavanadate in 250 ml hot H_2O, cool, and add 450 ml 70% $HClO_4$. Gradually add molybdate soln to vanadate soln with stirring, and dil. to 2 L.

(b) *Phosphate std soln.*—Prep. solns of pure, dry (2 hr at 105°) KH_2PO_4 (52.15% P_2O_5) contg 0.4–1.0 mg P_2O_5/ml in 0.1 mg increments. Prep. fresh solns contg 0.4 and 0.7 mg P_2O_5/ml weekly.

2.025 PREPARATION OF STANDARD CURVE

Pipet into 100 ml vol. flasks 5 ml aliquots of the 7 std phosphate solns (2–5 mg P_2O_5/aliquot) and add 45 ml H_2O. Then, within 5 min. for entire series, add 20 ml molybdovanadate reagent by buret or pipet, dil. to mark, and mix. Let stand 10 min.

Select 2 absorption cells (std and sample cells) and fill both with the 2 mg std. Set spectrophotometer to 400 mμ and adjust to zero absorbance with std cell. Sample cell must check zero absorbance within 0.001 unit; otherwise read absorbance difference for sample cell and correct subsequent readings. (Choose cell showing positive absorbance against the other as the sample cell so that this positive absorbance is always subtracted.) Using sample cell, det. absorbance of other stds with instrument adjusted to zero ab-

sorbance for the 2 mg std. After each detn empty and refill cell contg 2 mg std, to avoid error that might arise from temp. changes. Plot absorbance against concn in mg P_2O_5/ml std soln.

2.026 PREPARATION OF SOLUTION

Treat 1 g sample as in **2.018**(a), (b), (c), (d), or (e), preferably (e) when these acids are suitable solvent. (Soln should be free of N oxides and NOCl.)

(a) For P_2O_5 content up to 5%, dil. to 250 ml.

(b) For P_2O_5 content >5%, dil. to such vol. that 5 or 10 ml aliquot contains 2–5 mg P_2O_5.

2.027 DETERMINATION

Pipet, into 100 ml vol. flasks, 5 ml aliquots of std phosphate solns contg 2 and 3.5 mg P_2O_5/aliquot, resp., and develop color as in **2.025**. Adjust instrument to zero absorbance for 2 mg std and det. absorbance of 3.5 mg std. (It is essential that the absorbance of the latter std be practically identical with corresponding value on std curve.)

(a) *Samples containing up to 5% P_2O_5.*—Pipet, into 100 ml vol. flask, 5 ml sample soln, **2.026**(a), and 5 ml std phosphate soln contg 2 mg P_2O_5. Develop color and det. absorbance of this soln concurrently with and in same manner as for std phosphate solns in preceding par., with instrument adjusted to zero absorbance for 2 mg color std. Read P_2O_5 content of soln from std curve. With series of sample solns, empty and refill cell contg 2 mg std after each detn.

% P_2O_5 in sample = 100 × [(mg P_2O_5 from std curve − 2)/20].

(b) *Samples contg >5% P_2O_5.*—Pipet 5 or 10 ml sample soln, **2.026**(b), into 100 ml vol. flask. Without adding std phosphate soln, proceed as in (a).

% P_2O_5 in sample = 100 × (mg P_2O_5 from std curve/mg sample in aliquot).

Water-Soluble Phosphorus

2.028 *Gravimetric Method—Official*

Place 1 g sample on 9 cm filter and wash with small portions of H_2O until filtrate measures ca 250 ml. Let each portion pass thru filter before adding more and use suction if washing would not otherwise be complete within 1 hr. If filtrate is turbid, add 1–2 ml HNO_3, dil. to convenient vol., mix well, and proceed as in **2.019**.

2.029 *Volumetric Method—Official*

Treat sample as in **2.028**. To aliquot of soln corresponding to 0.1, 0.2, or 0.4 g, add 10 ml HNO_3, nearly neutralize with NH_4OH, dil. to 60 ml, and proceed as in **2.022**.

Citrate-Insoluble Phosphorus—Official

2.030 REAGENTS

(a) *Ammonium citrate soln* (*9*).—Should have sp. gr. of 1.09 at 20° and pH of 7.0 as detd electrometrically or colorimetrically with phenol red. When using colorimetric method proceed as follows:

Dissolve 370 g cryst. citric acid in 1.5 L H_2O and nearly neutralize by adding 345 ml NH_4OH (28–29% NH_3). If concn of NH_3 is <28%, add correspondingly larger vol. and dissolve citric acid in correspondingly smaller vol. H_2O. Cool, and make exactly neutral as follows:

Transfer 10 ml of the citrate soln to test tube of H-ion comparator set with color stds and add 0.5 ml 0.02% phenol red soln, **13.022** (1+1) (or vol. required to give same concn of indicator used in color stds). Add from graduated pipet few drops NH_4OH (1+7), mix, and compare color with that of color stds of same indicator, using comparator; add more NH_4OH if necessary, and repeat test until color matches that of color std corresponding to pH 7.0. If NH_4OH added exceeds that required to give pH 7.0, repeat test, using less NH_4OH. From quantity of NH_4OH required to produce in sample color that exactly matches std, calc. quantity of NH_4OH required to neutralize soln. Add this quantity of NH_4OH and check pH of soln by repeating test as before, adding small quantity of NH_4OH or of a citric acid soln, as may be required. When color matches, dil. soln, if necessary, to sp. gr. of 1.09 at 20°. (Vol. will be ca 2 L.) Keep in tightly stoppered bottles and check pH from time to time. (Phenol red is recommended in place of bromothymol blue because with latter indicator salt effect due to presence of NH_4 citrate soln gives pH reading ca 0.20 unit too high. If bromothymol blue is used, subtract 0.20 from observed reading.)

(b) *Other reagents and solns.*—*See* **2.017, 2.020,** or **2.024.**

2.031 DETERMINATION (*10*)

(a) *Acidulated samples and mixed fertilizers.*—After removing H_2O-sol. P_2O_5, **2.028**, transfer filter and residue, within 1 hr, to 200 or 250 ml flask contg 100 ml NH_4 citrate soln previously heated to 65°. Close flask tightly with smooth rubber stopper, shake vigorously until paper is reduced to pulp, and relieve pressure by removing stopper momentarily. Continuously agitate contents of stoppered flask in app. equipped to hold contents of flask at exactly 65°. (Action of app. should be such that dispersion of sample in citrate soln is continually maintained and entire inner surface of flask and stopper is continually bathed with soln.)

Exactly 1 hr after adding filter and residue,

remove flask from app. and immediately filter contents by suction as rapidly as possible thru Whatman No. 5 paper or equiv., using büchner or ordinary funnel with Pt or other cone. Wash with H_2O at 65° until vol. filtrate is ca 350 ml, allowing time for thoro draining before adding more H_2O. If material is one that will yield cloudy filtrate, wash with *5% NH_4NO_3 soln*. Det. P_2O_5 in citrate-insol. residue by one of following methods:

(1) Dry filter and contents, transfer to crucible, ignite until all org. matter is destroyed, and digest with 10–15 ml HCl until all phosphate dissolves; or (2) treat wet filter and contents as in **2.018**(**a**), (**c**), (**d**), or (**e**). Dil. soln to 200 ml, or other suitable vol., mix well, filter thru dry filter, and det. P_2O_5 as in **2.019, 2.022,** or **2.027.**

(**b**) *Non-acidulated samples.*—Place 1 g sample (ground to pass No. 35 sieve in case of Ca metaphosphate) on dry 9 cm paper. Without previous washing with H_2O, proceed as in (**a**). If sample contains much org. matter (bone, fish, etc.), dissolve residue insol. in NH_4 citrate as in **2.018**(**c**), (**d**), or (**e**).

2.032 Citrate-Soluble and Available Phosphorus—Official

Subtract sum of H_2O-sol. and citrate-insol. P_2O_5 from total P_2O_5 to obtain citrate-sol. P_2O_5. Subtract citrate-insol. P_2O_5 from total P_2O_5 to obtain available P_2O_5.

NITROGEN

2.033 Detection of Nitrates—Official

Mix 5 g sample with 25 ml hot H_2O, and filter. To 1 vol. of this soln add 2 vols H_2SO_4, free from HNO_3 and oxides of N, and let cool. Add few drops *concd $FeSO_4$ soln* in such manner that fluids do not mix. If nitrates are present, junction shows at first purple, afterwards brown, or if only minute quantity is present, reddish color. To another portion of soln add 1 ml *1% $NaNO_3$ soln* and test as before to det. whether enough H_2SO_4 was added in first test.

Total Nitrogen *(11)*

(Provide adequate ventilation in laboratory and do not permit accumulation of exposed Hg)

2.034 REAGENTS

(**a**) *Sulfuric acid.*—93–98% H_2SO_4, N-free.

(**b**) *Mercuric oxide or metallic mercury.*—HgO or Hg, reagent grade, N-free.

(**c**) *Potassium sulfate (or anhydrous sodium sulfate).*—Reagent grade, N-free.

(**d**) *Salicylic acid.*—Reagent grade, N-free.

(**e**) *Sulfide or thiosulfate soln.*—Dissolve 40 g commercial K_2S in 1 L H_2O. (Soln of 40 g Na_2S or 80 g $Na_2S_2O_3.5H_2O$ in 1 L may be used.)

(**f**) *Sodium hydroxide.*—Pellets or soln, nitrate-free. For soln, dissolve ca 450 g solid NaOH in H_2O and dil. to 1 L. (Sp. gr. of soln should be 1.36 or higher.)

(**g**) *Zinc granules.*—Reagent grade.

(**h**) *Zinc dust.*—Impalpable powder.

(**i**) *Methyl red indicator.*—Dissolve 1 g Me red in 200 ml alcohol.

(**j**) *Hydrochloric or sulfuric acid std soln.*—$0.5N$, or $0.1N$ when amount of N is small. Prep. as in **42.009** or **42.037.**

(**k**) *Alkali std soln.*—$0.1N$. Prep. as in **42.030–42.032.**

Stdze each std soln with primary std, Chap. **42,** and check one against the other. Test reagents before using by blank detn with 2 g sugar, which insures partial reduction of any nitrates present.

2.035 APPARATUS

(**a**) *For digestion.*—Use Kjeldahl flasks of hard, moderately thick, well-annealed glass with total capacity ca 500–800 ml. Conduct digestion over heating device adjusted to bring 250 ml H_2O at 25° to rolling boil in ca 5 min. To test heaters, preheat 10 min. if gas or 30 min. if elec. Add 3–4 boiling chips to prevent superheating.

(**b**) *For distillation.*—Use Kjeldahl or other suitable flask of 500–800 ml capacity, fitted with rubber stopper thru which passes lower end of efficient scrubber bulb or trap to prevent mechanical carryover of NaOH during distn. Connect upper end of bulb tube to condenser tube by rubber tubing. Trap outlet of condenser in such way as to insure complete absorption of NH_3 distd over into acid in receiver.

2.036 *Improved Kjeldahl Method for Nitrate-Free Samples—Official*

Place weighed sample (0.7–2.2 g) in digestion flask. Add 0.7 g HgO or 0.65 g metallic Hg, 15 g powd. K_2SO_4 or anhyd. Na_2SO_4, and 25 ml H_2SO_4. If sample >2.2 g is used, increase H_2SO_4 by 10 ml for each g sample. Place flask in inclined position and heat gently until frothing ceases (if necessary, add small amount of paraffin to reduce frothing); boil briskly until soln clears and then for at least 30 min. longer (2 hr for samples contg org. material).

Cool, add ca 200 ml H_2O, cool below 25°, add 25 ml of the sulfide or thiosulfate soln, and mix to ppt Hg. Add few Zn granules to prevent bumping, tilt flask, and add layer of NaOH (25 g solid reagent or enough soln to make contents strongly alk.) without agitation. (Thiosulfate or sulfide soln may be mixed with the NaOH soln before addn to flask.) Immediately connect flask to distg bulb on condenser, and, with tip of condenser immersed in std acid in receiver, rotate flask to mix contents thoroly; then heat until all NH_3 has

distd (at least 150 ml distillate). Titr. excess std acid in distillate with std alkali soln, using Me red. Correct for blank detn on reagents.

2.037 *Improved Kjeldahl Method for Nitrate-Containing Samples—Official*

Place weighed sample (0.7–2.2 g) in digestion flask. Add 40 ml H_2SO_4 contg 2 g salicylic acid. Shake until thoroly mixed and let stand, with occasional shaking, 30 min. or more; then add (1) 5 g $Na_2S_2O_3.5H_2O$ or (2) 2 g Zn dust (as impalpable powder, not granulated Zn or filings). Shake and let stand 5 min.; then heat over low flame until frothing ceases. Turn off heat, add 0.7 g HgO (or 0.65 g metallic Hg) and 15 g powd. K_2SO_4 (or anhyd. Na_2SO_4), and boil briskly until soln clears, then at least 30 min. longer (2 hr for samples contg org. material).

Proceed as in second par. of **2.036**.

Reduced Iron Method (12)—First Action

(Applicable to materials with high $Cl:NO_3$ ratio and to materials contg only water-sol. N)

2.038 REAGENTS

Reduced iron.—Powder, electrolytically reduced, N.F.

For other reagents, *see* **2.034**.

2.039 DETERMINATION

(**a**) *Dry, mixed fertilizers.*—Place 0.5–2.0 g sample in Kjeldahl flask and add 2–5 g reduced Fe (5 g is enough for 0.185 g NO_3). Add ca 25 ml H_2O, rotating flask at angle to wash down sample. Let stand 15 min., with occasional agitation, to insure complete soln of all sol. salts. While rotating flask, add 25 ml cold H_2SO_4 (1+1) and let stand until visible reaction ceases. (Use hood or vented digestion unit.) Add boiling chips and boil 15–20 min., but do not take to dryness. (For samples contg org. matter, use 50 ml cold H_2SO_4 (1+1), boil 15–20 min., add 0.7 g HgO, and heat again 30 min.) Cool, and proceed as in **2.036**, second par.

(**b**) *Ammonium nitrate, sodium nitrate, and fertilizer solns, including complete fertilizers and nitrogen solns.*—Weigh accurately ca 2.5 g sample in small beaker and transfer quantitatively to 250 ml vol. flask, dil. to vol. with H_2O, and mix. Transfer 25 ml aliquot to Kjeldahl flask, add 5 g reduced Fe and 45 ml cold H_2SO_4 (1+1), and continue as in (**a**).

Ammoniacal Nitrogen

2.040 *Magnesium Oxide Method—Official*

Place 0.7–3.5 g, according to NH_3 content of sample, in distn flask with ca 200 ml H_2O and 2 g or more of *carbonate-free MgO.* Connect flask to condenser by Kjeldahl connecting bulb, distill 100 ml liquid into measured quantity of std acid, **2.034(j)** and titr. with std alkali soln, **2.034(k)**, using Me red, **2.034(i)**.

2.041 *Formaldehyde Titration Method—Official*

(Applicable to NH_4NO_3 and to $(NH_4)_2SO_4$)

Weigh 7.004 or 14.008 g sample and dil. to 250 or 500 ml. Pipet 25 or 50 ml into 300–500 ml erlenmeyer (ca 1.5 g may be rapidly weighed and washed directly into flask). Add ca 1 ml 37% HCHO soln for each 0.1 g sample in aliquot. Dil. to 150–200 ml and let stand 5 min. Titr. with 0.25–0.50N NaOH, **2.034(k)**, using 5 drops phthln, until there is no perceptible color change at point of contact, or until proper shade of pink persists. (If electrometric titrn is preferred, titr. to ca pH 8.6.) Det. blank on the HCHO soln.

% ammoniacal N = net ml NaOH × normality × 1.4008/wt sample.

Nitrate and Ammoniacal Nitrogen

2.042 *Ferrous Sulfate-Zinc-Soda Method—Official*

(Not applicable in presence of org. matter, Ca cyanamide, and urea)

Place 0.35, 0.5, or 0.7 g sample in 600–700 ml flask and add 200 ml H_2O, 5 g powd. Zn, 1–2 g $FeSO_4.7H_2O$, and 50 ml NaOH soln (sp. gr. 1.33). Connect flask with distg app., distill, collect distillate in usual way in std acid, **2.034(j)**, and titr. with std alkali soln, **2.034(k)**, using Me red, **2.034(i)**. In analysis of nitrate salts dissolve 3.5 or 5.0 g in H_2O, dil. to 250 ml, and use 25 ml aliquot.

2.043 *Devarda Method (13)—Official*

(Not applicable in presence of org. matter, Ca cyanamide, and urea)

Place 0.35 or 0.5 g sample in 600–700 ml flask and add 300 ml H_2O, 3 g *Devarda alloy*, and 5 ml NaOH soln (42% by wt), pouring latter down side of flask so that it does not mix at once with contents. By means of Davisson (*14*) or other suitable scrubbing bulb that will prevent passing over of any spray, connect with condenser, tip of which always extends beneath surface of std acid in receiving flask. Mix contents of distg flask by rotating. Heat slowly at first and then at rate to yield 250 ml distillate in 1 hr. Collect distillate in measured quantity of std acid, **2.034(j)**, and titr. with std alkali soln, **2.034(k)**, using Me red, **2.034(i)**.

In analysis of nitrate salts, dissolve 3.5 or 5.0 g in H_2O, dil. to 250 ml, and use 25 ml aliquot.

Nitrate Nitrogen

2.044 *Robertson Method (15)—Official*

(Applicable in presence of Ca cyanamide and urea)

(**a**) Det. total N as in **2.037.**

(**b**) Det. H_2O-insol. N as in **2.047,** but use 2.5 g sample. Dil. filtrate to 250 ml.

(**c**) Det. ammoniacal N in 50 ml filtrate as in **2.040.**

(**d**) Place another 50 ml portion filtrate in 500 ml Kjeldahl flask and add 2 g $FeSO_4.7H_2O$ and 20 ml H_2SO_4. (If total N is >5%, use 5 g $FeSO_4.7H_2O$.) Digest over hot flame until all H_2O is evapd and white fumes appear, and continue digestion at least 10 min. to drive off nitrate N. If severe bumping occurs, add 10–15 glass beads. Add 0.65 g Hg, or 0.7 g HgO, and digest until all org. matter is oxidized. Cool, dil., add the K_2S soln, and complete detn as in **2.036.** Before distn add pinch of mixt. of Zn dust and granular "20-mesh" Zn to each flask to prevent bumping.

Total N (**a**) − H_2O-insol. N(**b**) = H_2O-sol. N.

H_2O-sol. N − N obtained in (**d**) = nitrate N.

Ammoniacal N + nitrate N = mineral N.

Total N − mineral N = org. N.

2.045 *Jones Modification of Robertson Method (16)—Official*

(Applicable when water-sol. N need not be detd)

Weigh 0.5 g sample into Kjeldahl flask, add 50 ml H_2O, and rotate gently. Add 2 g $FeSO_4.7H_2O$ and rotate. Add 20 ml H_2SO_4. Digest over hot flame. When H_2O evaps and white fumes appear, add 0.65 g Hg and proceed as in **2.036.** Cool, dil., and distill as usual. Total N − N thus found = nitrate N.

2.046 **Water-Insoluble Nitrogen in Cyanamide (*17*)—Official**

Weigh 2 g finely ground sample and place in mortar. Gradually add ca 70 ml H_2O while stirring with pestle, and grind thoroly. Transfer mixt. to beaker, washing out mortar with H_2O. Filter on 11 cm paper. When all cyanamide has been transferred to paper, wash with addnl 250 ml H_2O, draining each portion before adding more H_2O. Transfer paper and residue to digestion flask. Det. N in residue as in **2.036.**

2.047 **Water-Insoluble Organic Nitrogen—Official**

Place 1 or 1.4 g sample in 50 ml beaker, wet with alcohol, add 20 ml H_2O, and let stand 15 min., stirring occasionally. Transfer supernatant to 11 cm Whatman No. 2 paper in 60° long-stem funnel 2.5″ diam., and wash residue 4 or 5 times by decanting with H_2O at room temp. (20–25°). Finally transfer all residue to filter and complete washing until filtrate measures 250 ml. Det. N in residue as in **2.036.**

Nitrogen Activity

2.048 *Removal of Water-Soluble Nitrogen—Official*

(**a**) *Mixed fertilizers.*—Place quantity of sample equiv. to 50 mg H_2O-insol. org. N, **2.047,** on 11 cm paper wet with alcohol, and wash with H_2O at room temp. until filtrate measures 250 ml. If material is oily or does not wet readily with H_2O, wash with 5 ml alcohol and then with the H_2O. If necessary to use 4 g or more of material, weigh required quantity into small beaker, wet with alcohol, wash by decanting, finally transfer to filter, and finish extn as directed previously.

(**b**) *Raw materials.*—Place quantity of sample equiv. to 50 mg H_2O-insol. N, **2.047,** in small mortar; add ca 2 g powd. rock phosphate, mix thoroly, transfer to filter, wet with alcohol, and wash with H_2O at room temp. until filtrate measures 250 ml. If material is oily or does not wet readily with H_2O, wash with 5 ml alcohol and then with the H_2O.

2.049 *Determination of Water-Insoluble Organic Nitrogen Soluble in Neutral Permanganate—Official*

Using 25 ml tepid H_2O, transfer insol. residue from **2.048** to 400 ml beaker; add 1 g Na_2CO_3, mix, and add 100 ml *2% $KMnO_4$ soln.* Cover with watch glass and immerse 30 min. in steam or hot H_2O bath, keeping liquid level in beaker below that of H_2O in bath. Stir twice at 10 min. intervals. After 30 min. remove from bath, add immediately 100 ml cold H_2O, and filter thru heavy 15 cm folded paper. Wash with small quantities of cold H_2O until filtrate measures ca 400 ml. Det. N in residue and paper as in **2.036,** correcting for N contained in paper. N thus obtained is inactive H_2O-insol. org. N.

% N from **2.047** − % N found = % H_2O-insol. org. N sol. in neutral permanganate.

Determination of Water-Insoluble Organic Nitrogen Distilled from Alkaline Permanganate (18)—Official

2.050 REAGENTS

(**a**) *Potassium permanganate stock soln.*—Dissolve ca 51 g $KMnO_4$ in 1 L H_2O. Dissolve 0.5 g $Na_2C_2O_4$ in 300 ml H_2O and 10 ml H_2SO_4. Heat to 75–80° and titr. with the $KMnO_4$ soln, using Mohr pipet or all-glass buret to deliver the $KMnO_4$ soln. (235.88/ml $KMnO_4$ = concn $KMnO_4$ in g/L.) Adjust concn to 50 g/L, protect from light, and store at temp. >15°.

(**b**) *Sodium hydroxide stock soln.*—Dissolve 300 g NaOH in 1 L H_2O. Cool before using.

(**c**) *Alkaline permanganate soln.*—Mix equal vols of stock solns (**a**) and (**b**) and add 10 ml H_2O for each liter of soln that mixt. is calcd to make. Use soln immediately, as it is unstable.

2.051 DETERMINATION

Dry residue remaining after treatment of sample as in **2.048** at temp. not >80°, and transfer from filter to 500–600 ml Kjeldahl flask, loosening adhering particles by rubbing gently with stiff brush but avoiding transfer of portions of brush or of paper fibers. Add 20 ml H_2O, 15–20 small glass beads or fragments of pumice, drop of *mineral lubricating oil* weighing not >50 mg, and 100 ml of the alk. permanganate soln. Connect with upright condenser to lower end of which has been attached 100 ml graduated cylinder contg std acid, **2.034(j)**, and so arranged as to receive distillate below surface of acid or otherwise trapped so as to prevent loss of NH_3 fumes. Digest slowly 30 min. with very low flame, barely below distn point, using coarse wire gauze and asbestos paper between flask and flame. Gradually raise temp., and after all danger from frothing has passed, distill 95 ml in 60±5 min., controlling distn to obtain ca 24 ml distillate in each 15 min. period. Conduct first part of distn over bare flame, but use wire gauze 10 min. before completion to avoid breaking flask. Transfer distillate to erlenmeyer or beaker and titr. with std alkali, **2.034(k)**, using Me red, **2.034**(i). When tendency to froth is noticed, lengthen digestion period at beginning of distn. During digestion gently rotate flask occasionally, particularly if material tends to adhere to sides.

N thus obtained is active H_2O-insol. org. N. If it is <55% of total H_2O-insol. org. N present, prep. second portion of sample as in **2.048**. Dry residue at <80°, transfer from filter to Kjeldahl flask as directed above, and det. N as in **2.036**. Recalc. % active H_2O-insol. N on basis of quantity of H_2O-insol. N thus found.

To transfer washed sample from filter to flask before digesting with alk. permanganate, spread filter on metal disk bent to form trough that fits palm of hand, brush larger portion of material into flask with spatula, and wash in remainder with 20 ml H_2O from 20 ml pipet or small wash bottle. Do not add more H_2O before digestion with the alk. permanganate, but otherwise proceed as with transfer of dried material.

Nitrogen Activity Index (AI) of Urea-Formaldehyde Compounds (*19*)—First Action

(Applicable to urea-formaldehyde compounds and mixts contg such compds)

2.052 REAGENT

Phosphate buffer soln.—0.063*M*. Dissolve 14.3 g KH_2PO_4 and 91.0 g K_2HPO_4 in 1 L H_2O. Dil. 100 ml to 1 L; pH of dild soln should be 7.5.

2.053 DETERMINATION

(**a**) Crush sample (do not grind) to pass No. 20 sieve.

(**b**) Det. cold H_2O-insol. N (*WIN*) by **2.047**. Ext. with H_2O at 25±2°.

(**c**) Det. hot H_2O-insol. N (*HWIN*) in the phosphate buffer soln. Place accurately weighed sample contg 0.1200 g *WIN* in 200 ml tall form beaker. Add 100 ml of the boiling buffer soln, stir, cover, and immerse in boiling H_2O bath so that liquid in beaker is below H_2O line in bath. Hold bath at rolling boil. Stir soln gently for ca 5 sec. at 10 min. intervals. After 30 min., remove from bath and filter at once thru 15 cm Whatman No. 12 fluted paper. If filtration takes >4 min., discard detn. Repeat detn, adding, with stirring, 1 g Celite filter-aid to hot extn mixt. just before removing beaker from bath. (Do not add Celite at earlier stage.) Adhere to boiling and filtration times with aid of stop watch. Wash the insol. residue onto paper with near-boiling H_2O, and continue washing residue from top down until total wash H_2O is 75–100 ml. Det. total N (*HWIN*) in the wet paper and residue as in **2.036**.

$$\textit{Activity index} = (\%\ WIN - \%\ HWIN) \times 100/\%\ WIN$$

Urea (*20*)—Official

2.054 REAGENT

Neutral urease soln.—Shake 1 g jack bean meal with 100 ml H_2O 5 min. Transfer 10 ml soln to 250 ml erlenmeyer, dil. with 50 ml H_2O, and add 4 drops Me purple. Titr. with 0.1*N* HCl to reddish-purple; then back-titr. to green with 0.1*N* NaOH. From difference in ml, calc. amount of 0.1*N* HCl required to neutralize remainder of soln (usually ca 2.5 ml/100 ml), add this amount of acid, and shake well.

2.055 DETERMINATION

Weigh 10±0.01 g sample and transfer to 15 cm Whatman No. 12 fluted filter paper. Leach with ca 300 ml H_2O into 500 ml vol. flask. Add 75–100 ml satd $Ba(OH)_2$ soln to ppt phosphates. Let settle and test for complete pptn with few drops satd $Ba(OH)_2$ soln. Add 20 ml 10% Na_2CO_3 soln to ppt excess Ba and any sol. Ca salts. Let settle and test for complete pptn. Dil. to vol., mix, and filter thru 15 cm Whatman No. 12 fluted paper. Transfer 50 ml aliquot (equiv. to 1 g sample) to 200 or 250 ml erlenmeyer and add 1–2 drops of

Me purple indicator. Acidify soln with $2N$ HCl and add 2–3 drops excess. Neutralize soln with $0.1N$ NaOH to first change in color of indicator. Add 20 ml neutral urease soln, close flask with rubber stopper, and let stand 1 hr at 20–25°. Cool flask in ice-H_2O slurry and titr. at once with $0.1N$ HCl to full purple color, then add ca 5 ml excess. Record total vol. added. Back-titr. excess HCl with $0.1N$ NaOH to neutral end point.

% Urea = (ml $0.1N$ HCl − ml $0.1N$ NaOH) ×0.3003/wt sample.

Biuret (*21*)—First Action

2.056 REAGENTS

(**a**) *Alkaline tartrate soln.*—Dissolve 40 g NaOH in 500 ml H_2O, cool, add 50 g $NaKC_4H_4O_6.4H_2O$, and dil. to 1 L. Let stand 1 day before use.

(**b**) *Copper sulfate soln.*—Dissolve 15 g $CuSO_4$ $.5H_2O$ in CO_2-free H_2O and dil. to 1 L.

(**c**) *Biuret std soln.*—1 mg/ml. Dissolve 100 mg reagent grade biuret in CO_2-free H_2O and dil. to 100 ml.

(**d**) *Ion exchange resin.*—Fill 50 ml buret with 30 cm column of Amberlite IR120(H) resin on glass wool plug. Regenerate column after each use by passing 100 ml H_2SO_4 (1+9) or HCl (1+4) thru column at ca 5 ml/min. and then washing with H_2O until pH of effluent is >6.

2.057 PREPARATION OF STANDARD CURVE

Transfer series of aliquots, 2–50 ml, of std biuret soln to 100 ml vol. flasks. Adjust vol. to ca 50 ml with CO_2-free H_2O, add 1 drop Me red, and neutralize with $0.1N$ H_2SO_4 to pink color. Add with swirling 20 ml alk. tartrate soln and then 20 ml $CuSO_4$ soln. Dil. to vol., shake 10 sec., and place in H_2O bath 15 min. at 30±5°. Also prep. reagent blank. Det. absorbance of each soln against blank at 555 mμ (instrument with 500–570 mμ filter is also satisfactory) with 2–4 cm cell. Plot std curve.

2.058 DETERMINATION

(**a**) *In urea.*—Stir continuously 2–5 g sample in 100 ml ca 50° H_2O 30 min. Filter and wash into 250 ml vol. flask, and dil. to vol. Transfer 25 ml aliquot to 100 ml vol. flask and proceed as in **2.057.**

(**b**) *In mixed fertilizers.*—Stir continuously 10–20 g sample in 150 ml ca 50° H_2O 30 min. Filter and wash into 250 ml vol. flask, and dil. to vol. Transfer 25 ml aliquot to column, **2.056**(**d**), and adjust flow to 4–5 ml/min. Receive eluate in 100 ml beaker. When liquid level falls to top of resin bed, wash with two 25 ml portions H_2O. To eluate and washings add 2 drops Me red and then $1N$ NaOH to yellow color. Add $0.1N$ H_2SO_4 until soln just turns pink, transfer to 100 ml vol. flask, and dil. to vol. with CO_2-free H_2O. Transfer 50 ml aliquot to 100 ml vol. flask and proceed as in **2.057.**

POTASSIUM

Lindo-Gladding Method (*22*)—*Official*

2.059 REAGENTS

(**a**) *Ammonium chloride soln.*—Dissolve 100 g NH_4Cl in 500 ml H_2O, add 5–10 g pulverized K_2PtCl_6, and shake at intervals 6–8 hr. Let mixt. settle overnight and filter. (Residue may be used to prep. fresh supply.)

(**b**) *Platinum soln.*—Use Pt soln contg equiv. of 0.05 g Pt (0.105 g H_2PtCl_6)/ml. 1 ml = 0.024 g K_2O.

(**c**) *Diglycol stearate soln.*—Dissolve 20 g diglycol stearate, tech., in 1 L benzene-alcohol (1+1).

(**d**) *Acid-alcohol.*—Mix 200 ml alcohol with 20 ml HCl and cool to room temp.

2.060 PREPARATION OF SOLUTION

(**a**) *Mixed fertilizers.*—Place 2.5 g sample, or factor wt 2.425 g, in 250 ml vol. flask, and add 125 ml H_2O and 50 ml satd $(NH_4)_2C_2O_4$ soln; add 1 ml of the diglycol stearate soln if needed to prevent foaming. Boil 30 min., add slight excess of NH_4OH, and after cooling, dil. to 250 ml. Mix, and pass thru dry filter.

(**b**) *Potassium salts* (*potassium chloride and sulfate, potassium-magnesium sulfate, and kainit*).—Dissolve 2.5 g, or factor wt 2.425 g, and dil. to 250 ml without adding NH_4OH and $(NH_4)_2C_2O_4$. When interfering substances such as NH_3, Ca, Al, etc., are present, proceed as in (**a**).

(**c**) *Organic materials* (*cottonseed meal, tobacco stems, etc.*).—For total K, sat. 10 g sample with H_2SO_4 and ignite in muffle at *low red heat* to destroy org. matter. Add little HCl, warm slightly to loosen mass from dish, transfer to 500 ml vol. flask, add NH_4OH and satd $(NH_4)_2C_2O_4$ soln, cool, dil. to 500 ml, mix, pass thru dry filter, and proceed as in **2.061**(**a**).

(**d**) *Ashes from wood, cotton hulls, etc.*—Boil 10 g sample with 300 ml H_2O 30 min., and add to hot soln slight excess of NH_4OH and then enough satd $(NH_4)_2C_2O_4$ soln to ppt all Ca present. Cool, dil. to 500 ml, mix, pass thru dry filter, and proceed as in **2.061**(**a**).

(**e**) *Potassium nitrate or potassium and sodium nitrate.*—If impure, proceed as in (**a**); if sufficiently pure, proceed as for potassium salts (**b**), except evap. aliquot to dryness in porcelain dish with 2 ml HCl (if Pt dish is used, add H_2SO_4 instead) and take up with H_2O and few drops HCl, before adding the Pt soln.

2.061 DETERMINATION

(**a**) *Mixed fertilizers.*—In ca 100 ml quartz, SiO_2, or Pt dish, evap. nearly to dryness 25 or 50

ml aliquot of soln, **2.060(a)**, (**c**), or (**d**), to which is added enough K-free 1*N* NaOH (1–2 ml) to prevent formation of free H_3PO_4 during ignition; add 1 ml H_2SO_4 (1+1) and 6–8 granules of granulated *sugar*, evap. to dryness, and ignite to white ash at low temp. (The H_2SO_4 may be added after evapn to dryness and before ignition.) Maintain dull red heat until residue is perfectly white. Dissolve residue in hot H_2O, using at least 20 ml/100 mg K_2O present, and add few drops HCl and then excess of the Pt soln. Evap. on H_2O bath to thick paste, avoiding exposure to NH_3. Treat residue with ca 6 ml of the acid-alcohol. (Temp. of wash solns should not be >30°.) After 15 min. filter on gooch or on medium fritted crucible (Pyrex M porosity), and wash ppt thoroly with alcohol, both by decanting and on filter, continuing washing after filtrate is colorless (75 ml is usually enough). Wash 5 or 6 times with 10 ml portions of the NH_4Cl soln to remove impurities from ppt. Wash again thoroly with alcohol and dry ppt 30 min. at 100°. Cool and weigh. Wash K_2PtCl_6 thru crucible with hot H_2O; then wash all H_2O from crucible with alcohol and dry crucible and residue 30 min. at 100°. Cool, reweigh, and calc. wt difference to K_2O. If factor wt and 50 ml aliquot (contg 0.485 g sample) are used, multiply wt by 40 to obtain % K_2O.

(**b**) *Potassium chloride.*—Acidify 50 ml soln prepd as in **2.060(b)** with few drops HCl, add excess of the Pt soln, and evap. to thick paste. Treat residue as in (**a**). If NH_4OH and $(NH_4)_2C_2O_4$ are used in prepg soln, ignite and complete detn as in (**a**).

(**c**) *Potassium sulfate, potassium-magnesium sulfate, and kainit.*—Acidify 50 ml soln prepd as in **2.060(b)** with few drops HCl and add excess of the Pt soln. Evap. mixt. and proceed as in (**a**), but use 25 ml portions of the NH_4Cl soln. If NH_4OH and $(NH_4)_2C_2O_4$ are used in prepg soln, ignite and complete detn as in (**a**), but use 25 ml portions of the NH_4Cl soln.

To convert K_2PtCl_6 to KCl use factor 0.3068; to K_2SO_4, 0.3585; to K_2O, 0.1938.

Wet-Digestion Method (23)—Official

2.062 PREPARATION OF SOLUTION

Proceed as in **2.060(a)**.

2.063 DETERMINATION

Place 50 ml aliquot soln (or 25 ml aliquot and 25 ml H_2O, if sample contains >20% K_2O) in 500 ml Kjeldahl flask. Add 10 ml HNO_3 and *silica granule* (ca 1 cm long, previously weighed along with prepd gooch or medium porosity fritted Pyrex crucible). Boil 2 min. and add 10 ml HCl. Boil down to ca 25 ml and add 5 ml HCl and excess Pt soln. Boil down to 10–15 ml, rotating flask occasionally, and then add 5 ml HCl. Reduce heat and boil down to 3–5 ml (depending on amount of ppt), rotating flask frequently near end of evapn. Remove flask from heat and swirl to dissolve any sol. residue on walls. Cool and immediately add 25 ml alcohol so that it washes down neck of flask. Chill under tap, swirl, and let stand at least 5 min. Decant into tared crucible and transfer ppt and granule with aid of stream of alcohol. Wash 5–6 times with 10 ml portions NH_4Cl soln, **2.059(a)**, to remove Mg and Na salts from ppt. Wash again thoroly with alcohol and dry ppt 30 min. at 100°. Weigh and subtract wt crucible plus silica granule. $K_2PtCl_6 \times 0.1938 = K_2O$.

Recovery of Platinum (24)—Procedure

2.064 RECOVERY FROM ALCOHOL WASHINGS

(**a**) Let NH_4Cl washings run into flask with the alcohol washings. Let the $(NH_4)_2PtCl_6$ settle, decant supernatant, and save residue. Reduce as in **2.065(a)** or (**b**).

(**b**) Evap. the alcohol waste in porcelain dish on steam bath or elec. hot plate. (Piece of filter paper in dish prevents most of Pt from sticking to dish.) Filter on büchner and wash the reduced Pt. Transfer to porcelain dish and ignite at ca 700° in muffle ca 20 min. Digest reduced Pt in porcelain dish on steam bath with several portions of HCl (1+3). Repeat until soln is colorless. Wash well with H_2O until test with $AgNO_3$ shows no Cl. Digest with few portions of HNO_3 (1+4), wash, dry, and weigh.

(**c**) Acidify the alcohol waste with HCl. Add either "20-mesh" Zn, or Al in stick or sheet form (for 75–150 ml acid use 10–20 g metal), and let stand until all Pt is reduced. Filter, ignite at 700°, and proceed as in (**b**).

2.065 RECOVERY FROM K_2PtCl_6 SALT

(**a**) Dissolve the K_2PtCl_6 in 20 parts or more of hot H_2O, acidify with HCl, and reduce with either "20-mesh" Zn or Al in sheet or stick form. Filter and ignite as in **2.064(b)**.

(**b**) Dissolve the K_2PtCl_6 in H_2O and ppt as $(NH_4)_2PtCl_6$ with NH_4Cl. Let stand several hr, filter on büchner with suction, and wash with alcohol. Transfer to porcelain dish and ignite in muffle, first at ca 200° ca 20 min. and finally 30 min. at ca 700°.

(**c**) Dissolve the K_2PtCl_6 in 20 parts or more of boiling H_2O. Add *Na formate* slowly (pinch at time), stirring well at each addn. (Use great care to control excessive foaming with resultant loss of Pt.) Reduction is complete when soln becomes colorless. If supernatant does not become colorless, test for complete reduction as follows:

Pipet 25 ml into 250 ml beaker, and add few

drops HCl and small quantity of KI soln. Red color indicates presence of unreduced Pt (or other oxidant such as HNO_3).

Filter the reduced Pt and ignite as in **2.064(b)**.

2.066 PREPARATION OF PLATINUM SOLUTION

Dissolve Pt from **2.064** or **2.065** in porcelain dish on steam bath with 3 parts HCl and 1 part HNO_3. Evap. with addns of HCl 3 times to remove excess of HNO_3, and then with H_2O 3 times to remove excess of HCl, but do not evap. below $\frac{1}{4}$ original vol. Filter, and dil. to calcd vol. Evap. and test 10 ml portion, or portion equiv. to 1 g Pt, for material insol. in 80% alcohol. If impurities are evident, reduce soln again, purify Pt, and redissolve. To det. concn of the soln, evap. 2 ml in porcelain dish with ca 0.5 g excess of K_2SO_4. Add alcohol and wash K_2PtCl_6 as in **2.061.** (Soln may be made up so that 1 ml = 1% K_2O in 1 g sample.)

Flame Photometric Method (25)—Official

2.067 REAGENTS AND APPARATUS

(**a**) *Ammonium carbonate soln.*—Dissolve 50 g $(NH_4)_2CO_3$ in 1 L H_2O.

(**b**) *Methyl red indicator.*—Dissolve 0.2 g Me red in 100 ml alcohol.

(**c**) *Dilute nitric acid.*—Reagent grade, 1+10.

(**d**) *Anion exchange resin.*—Amberlite IR-4B (Fisher Scientific Co., Pittsburgh, Pa.); Duolite A-7 or Duolite A-41 (Resinous Products Co., Redwood City, Calif.); De-Acidite or Permutit-S (Permutit Inc., 50 West 44th St., New York 36, N. Y.); or equiv.

(**e**) *Potassium nitrate or potassium chloride.*—Recrystallize reagent grade salt twice from H_2O and dry 5 hr at 105°.

(**f**) *Ion exchange column.*—Made from 12" length of std wall glass tubing, 2.5 cm o.d.; one end closed by 1-hole No. 4 rubber stopper thru which is inserted 2-way stopcock or glass tubing connected to rubber tubing and compressor clamp. Do not let stopcock tubing protrude above stopper. Choose stopper large enough so that there is no space between stopper vertex and column wall. Place glass wool plug in bottom of column and fill with H_2O suspension of resin to height of 8". Regenerate resin after 10 successive aliquots have passed thru, except Amberlite IR-4B which can be used for 20 aliquots.

2.068 PREPARATION OF RESIN

Place ca 1.5 lb resin in 4 L beaker and add enough 5% NaOH to completely float resin (2–3 L) while it is being stirred. Stir 30 min. with elec. stirrer. Let resin settle, and decant NaOH soln. Repeat treatment with 5% NaOH 2 more times, decanting NaOH soln after final treatment. Add 3 L H_2O to resin, stir few min., let resin settle, and decant wash H_2O. Repeat 3–4 times. Resin is now in free base form. Regenerate to NO_3 form by treating 3 times with 5% HNO_3, using same technique as with NaOH soln. Wash resin until washings reach pH 2 or above by back-washing in column or by stirring and decanting in large beaker. Keep resin damp during storage.

2.069 PREPARATION OF SOLUTION

(**a**) *Mixed fertilizers and potassium-magnesium sulfate.*—Weigh 1.5058 g sample into 250 ml flask (500 ml flask if sample contains >30% K_2O), add 100 ml H_2O and 20 ml $(NH_4)_2CO_3$ soln, and boil 5 min. Cool, dil. to vol., mix, and pass thru dry filter.

(**b**) *Potassium chloride and sulfate.*—Dissolve 1.5058 g in H_2O and dil. to 500 ml.

2.070 PREPARATION OF STANDARD CURVE

Dissolve 1.2931 g KNO_3 (or 0.9535 g KCl) in H_2O and dil. to 500 ml (1000 ppm K). Prep. std solns by diln covering range 0–80 ppm K at intervals not >10 ppm, adding appropriate amount of $LiNO_3$ if internal std instrument is to be used. Prep. std curve of emission against concn, adjusting instrument so that 50 ppm K gives reading near mid-scale.

2.071 DETERMINATION

(**a**) *Mixed fertilizers, potassium sulfate, and potassium-magnesium sulfate.*—Transfer 10 ml aliquot of sample soln to 250 ml beaker. Add 1 drop Me red and neutralize with HNO_3 (1+10). Adjust H_2O level in column to top of resin and transfer aliquot to column quantitatively. Open stopcock to give flow rate of 2 drops/sec., collecting effluent in 250 ml vol. flask. Wash aliquot into resin with 2–3 small portions of H_2O. Collect 50–75 ml effluent; then open stopcock and collect addnl 100 ml by pouring H_2O into column, making certain that H_2O level does not fall below top of resin bed. Dil. to vol. and mix (if internal std instrument is used, add required amount of $LiNO_3$ before dilg to vol.). Det. ppm K from std curve. (Temp. of std and sample solns must not differ by >2°.) Calc. % K_2O as follows:

$$0\text{–}30\%: \text{ppm K}/2 = \%\ K_2O$$
$$>30\%: \text{ppm K}/1 = \%\ K_2O$$

(**b**) *Potassium chloride.*—Proceed as in (**a**) but omit neutralization and resin treatment.

2.072 INSTRUMENT AND PROCEDURE PERFORMANCE TEST

Weigh 1.5058 g K acid phthalate (primary std) and transfer to 250 ml vol. flask. Add ca 0.5 g $(NH_4)_2HPO_4$ and proceed as in **2.069(a)**, beginning "add 100 ml H_2O . . . " Calcd % K_2O = 23.0.

Volumetric Sodium Tetraphenylboron Method (26)—Official

2.073 REAGENTS

(a) *Sodium hydroxide soln.*—20%. Dissolve 20 g NaOH in 100 ml H_2O.

(b) *Formaldehyde soln.*—37%.

(c) *Sodium tetraphenylboron (STPB) soln.*—Approx. 1.2%. Dissolve 12 g $NaB(C_6H_5)_4$ (J. T. Baker Chemical Co., Phillipsburg, N. J.) in ca 800 ml H_2O. Add 20–25 g $Al(OH)_3$, stir 5 min., and filter (Whatman No. 42 paper or equiv.) into 1 L vol. flask. Rinse beaker sparingly with H_2O and add to filter. Collect entire filtrate, add 2 ml 20% NaOH, dil. to vol. with H_2O, and mix. Let stand 48 hr and stdze. Adjust so that 1 ml STPB =1% K_2O. Store at room temp.

(d) *Quaternary ammonium chloride soln.*—Approx. 0.625%. Dil. 50 ml 12.8% Zephiran chloride (Winthrop-Stearns, Inc., New York 18, N.Y.; also available at local pharmacies) to 1 L with H_2O, mix, and stdze. Cetyltrimethylammonium bromide may be substituted for Zephiran chloride.

(e) *Clayton Yellow (Titan Yellow; Color Index No. 19540).*—0.04%. Dissolve 40 mg in 100 ml H_2O.

2.074 STANDARDIZATION OF SOLUTIONS

(a) *Zephiran chloride.*—To 1.00 ml STPB soln in 125 ml erlenmeyer, add 20–25 ml H_2O, 1 ml 20% NaOH, 2.5 ml HCHO, 1.5 ml 4% $(NH_4)_2C_2O_4$, and 6–8 drops of indicator, (e). Titr. to pink end point with Zephiran chloride soln, using 10 ml semimicro buret. Adjust Zephiran chloride soln so that 2.00 ml = 1.00 ml STPB soln.

(b) *Sodium tetraphenylboron soln.*—Dissolve 2.500 g KH_2PO_4 in H_2O in 250 ml vol. flask, add 50 ml 4% $(NH_4)_2C_2O_4$ soln, dil. to vol. with H_2O, and mix. (It is not necessary to bring to boil.) Transfer 15 ml aliquot (52.87 mg K_2O, 43.88 mg K) to 100 ml vol. flask; add 2 ml 20% NaOH, 5 ml HCHO, and 43 ml STPB reagent. Dil. to vol. with H_2O, mix *thoroly*, let stand 5–10 min., and pass thru dry filter. Transfer 50 ml aliquot of filtrate to 125 ml erlenmeyer, add 6–8 drops of indicator, (e), and titr. excess reagent with Zephiran soln. Calc. titer as follows:

$F = 34.58/(43 \text{ ml} - \text{ml Zephiran}) = \%\ K_2O/\text{ml}$ STPB reagent. Factor F applies to all fertilizers if 2.5 g sample is dild to 250 ml and 15 ml aliquot is taken for analysis. If results are to be expressed as K rather than as K_2O, substitute 28.70 for 34.58 in calcg F.

2.075 DETERMINATION

Place 2.5 g (1.25 g if $K_2O > 50\%$) sample in 250 ml vol. flask, add 50 ml 4% $(NH_4)_2C_2O_4$ and 125 ml H_2O, and boil 30 min. Cool, dil. to vol. with H_2O, mix, and pass thru dry filter or let stand until clear. Transfer 15 ml aliquot of sample soln to 100 ml vol. flask and add 2 ml 20% NaOH and 5 ml HCHO. Add 1 ml std STPB soln for each 1% K_2O expected in sample plus addnl 8 ml excess to insure complete pptn. Dil. to vol. with H_2O, mix *thoroly*, let stand 5–10 min., and pass thru dry filter (Whatman No. 12 or equiv.). Transfer 50 ml filtrate to 125 ml erlenmeyer, add 6–8 drops of indicator, (e), and titr. excess reagent with std Zephiran soln.

% K_2O in sample = (ml STPB added − ml Zephiran) $\times F$, where $F = \%\ K_2O$/ml STPB reagent. (Multiply by 2 if 1.25 g sample was used.)

OTHER ELEMENTS

Acid-Soluble Boron (*27*)—Official

2.076 APPARATUS

Use high sensitivity glass electrode pH meter for titrn. (Quinhydrone electrode system or similar assemblies may also be used.) Use assembly with burets, electrodes, and motor-driven stirrer, arranged for convenient use with 250 ml beaker. Use ordinary 50 ml burets for the 0.025*N* NaOH and 0.02*N* HCl.

2.077 REAGENTS

(a) *Boric acid std soln.*—Dissolve 1 g H_3BO_3 in H_2O and dil. to 1 L. 1 ml = 0.1750 mg B.

(b) *Sodium hydroxide std soln.*—CO_2-free, ca 0.025*N*. Stdze as follows: Pipet 25 ml of the std H_3BO_3 soln into 250 ml beaker, add 3.0 g NaCl, acidify to Me red, dil. to 150 ml, boil to expel CO_2, cool, and titr. potentiometrically as in **2.078.** Det. titrn blank by repeating procedure, substituting 25 ml H_2O for the H_3BO_3 soln. Calc. B equivalence as follows:

mg B/ml = 4.374/[(ml NaOH soln) − (ml blank)].

Protect from atmospheric CO_2 by soda-lime tubes or other suitable means.

(c) *Methyl red indicator.*—Dissolve 0.1 g Me red in 50 ml alcohol, dil. to 100 ml with H_2O, and filter if necessary.

2.078 DETERMINATION

Weigh sample within 1 mg (1.0 g for up to 0.45% B, smaller samples for above that content) and place in 250 ml beaker. Add ca 50 ml H_2O and 3 ml HCl. Heat to boiling and keep hot until carbonates are decomposed. Keep soln hot but do not boil during following treatment to remove phosphate:

Add *10% $Pb(NO_3)_2$ soln*, usually 10 ml, or 1 ml for each 1.2% P_2O_5 if P_2O_5 content is known to be >12%. Add $NaHCO_3$, little at time, until soln approaches neutrality (often observed by formation of white ppt in addn to insol. matter already

present). Add few drops Me red and continue adding $NaHCO_3$ gradually until *just* alk. to Me red (yellow or very slightly orange). Keep mixt. hot but not boiling (H_2O bath or steam bath is best) 30 min., adding addnl small quantities of $NaHCO_3$ if needed to keep same indicator color. (If indicator is bleached by nitrate present, add more; if color is obscured by org. matter, use external spot tests to follow neutralization.) After neutralization and heating, 40–50 ml soln should remain.

Filter hot soln into 250 ml beaker and wash solids thoroly with hot H_2O. Acidify filtrate with few drops HCl and boil briefly to expel most of CO_2. Neutralize hot soln with 0.5*N* NaOH, and reacidify with 0.5*N* HCl, using 0.3–0.5 ml in excess. Dil. to ca 150 ml and boil gently few min. to expel remaining CO_2. Cool to room temp. in running H_2O. Roughly neutralize mixt., using CO_2-free 0.5*N* NaOH, and place beaker in titrn assembly with electrodes and stirrer immersed. Start stirrer and adjust pH to exactly 6.30 by adding the 0.025*N* NaOH or 0.02*N* HCl as required. (When properly adjusted, pH should be steady; drifting usually is due to incomplete removal of CO_2.) When reading of pH 6.30 is steady, read the 0.025*N* NaOH buret, add 20 g *mannitol* or cryst. D-*sorbitol*, and titr. with the 0.025*N* NaOH to pH 6.30. (Conveniently done with slide-wire type instrument by opening pH meter circuit when mannitol is added, leaving scale setting at 6.30, closing circuit again when indicator color shows that end point is being approached, and carefully adding the std NaOH soln until galvanometer needle returns to zero. With practice, somewhat slow approach to equilibrium, characteristic of glass electrode, can be anticipated so as not to overrun end point.) When end point is reached, again read buret. Correct quantity of std NaOH soln used by reagent blank detd by repeating detn with all reagents but without sample. Calc. B content by following formula:

$$\% \text{ B} = (\text{ml NaOH soln in detn} - \text{ml blank}) \times (\text{mg B/ml NaOH soln})/(10 \times \text{g sample})$$

2.079 Water-Soluble Boron (*28*)—Official

Weigh 2.5 g sample into 250 ml beaker. Add 125 ml H_2O, boil gently ca 10 min., and filter hot thru Whatman No. 40 paper into 400 ml beaker. Wash solids well with 6 washings hot H_2O and make vol. to at least 200 ml with H_2O. Heat filtrate just to boiling. Add 15 ml 10% $BaCl_2$ soln to ppt sulfates and phosphates, and add powd. $Ba(OH)_2$, cautiously with stirring, until just alk. to phthln, avoiding large excess. Boil in open beaker at least 60 min. to expel NH_3. (Samples colored by org. matter should be boiled longer.) If necessary, add H_2O to keep vol. to at least 150 ml. Add and stir 1–2 teaspoonfuls Filter-Cel or other inert filtering aid, and filter with suction thru packed paper pads into 500 ml Pyrex erlenmeyer. Wash ppt 6 times with hot boiled H_2O. (Avoid too large wash vols which increase vol. in flask to point of dangerous bumping in next step.)

Make filtrate just colorless to phthln with HCl (1+5), add Me red, and make just pink with the acid. Add 5 or 6 boiling stones and stirring rod, cover with watch glass, and boil 5 min. to remove CO_2. Cool in cold H_2O while covered. Wash cover glass, stirrer, and sides of flask. Titr. to yellow of Me red with std 0.05*N* NaOH, **42.030-42.034.** Add 20 g D-*mannitol* and 1 ml or more phthln, shake, and wash down sides of flask. Titr. to pink end point. Det. blank in exactly same manner as sample. 1 ml 0.05*N* NaOH = 0.000540 g B or 0.00477 g $Na_2B_4O_7.10H_2O$. Or, (Titer − blank) × factor = lbs $Na_2B_4O_7.10H_2O$/ton (factor = 3.807 for 0.05*N* NaOH).

Carbonate Carbon (*29*)—Official

2.080 APPARATUS AND REAGENTS

Knorr alkalimeter with CO_2 absorption train.—Fill guard tube of alkalimeter with Ascarite. Connect upper end of condenser to absorption train consisting of 5 or 6 U-shape, g-s. drying tubes (or equiv.) joined in series. Charge first tube with H_2SO_4 and second with Ag_2SO_4-H_2SO_4 soln (10 g Ag_2SO_4 in 100 ml H_2SO_4) to remove acidic gases other than CO_2. Charge third tube with $Mg(ClO_4)_2$ to absorb H_2O. Fill inlet ⅔ of fourth and succeeding tubes with Ascarite to absorb CO_2, and outlet ⅓ of each tube with $Mg(ClO_4)_2$. Connect last tube in train with aspirating bottle or suction source.

Condition app. before use each day, and also when freshly filled tube is placed in train, by aspirating air at rate of 2–3 bubbles/sec. thru the dry alkalimeter assembly and absorption train until CO_2 absorption tubes attain constant wt (usually 20–30 min.). Use similarly packed tare and std procedure for wiping tubes with dry lint-free cloth before each weighing.

2.081 DETERMINATION

Transfer 2 g sample to dry alkalimeter flask. Momentarily open stopcocks of first 2 CO_2 absorption tubes to air to equalize pressure, weigh tubes separately, and place in position in train. With assembled alkalimeter connected to absorption train, adjust rate of aspiration of air thru system to ca 2 bubbles/sec. Close funnel stopcock, remove alkalimeter guard tube, fill funnel with 50 ml HCl (1+4), and replace guard tube. Open funnel stopcock and let acid run slowly into flask, taking care that evolution of gas is so gradual as not to materially increase flow thru tubes. After all acid is added, agitate alkalimeter assembly to insure complete dispersion of sample in the acid

soln. Continue aspiration, gradually heat contents of flask to boiling, and boil 2–3 min. after H_2O begins to condense. Discontinue heating and continue aspiration 15–20 min. or until app. cools. Remove, equalize internal and external pressure, and reweigh absorption tubes.

Increase in wt = wt CO_2. (Material increase in wt of second tube usually indicates exhaustion of first tube, but may result from too rapid evolution of CO_2 in relation to aspiration rate.) Report % (by wt) CO_2 in sample.

Water-Soluble-Chlorine (*30*)—Official

2.082 REAGENTS

(a) *Silver nitrate std soln.*—Dissolve ca 5 g recrystd $AgNO_3$ in H_2O and dil. to 1 L. Stdze against pure, dry NaCl and adjust so that 1 ml soln = 0.001 g Cl.

(b) *Potassium chromate indicator.*—Dissolve 5 g K_2CrO_4 in 100 ml H_2O.

2.083 DETERMINATION

Place 2.5 g sample on 11 cm filter paper and wash with successive portions of boiling H_2O until washings total nearly 250 ml, collecting filtrate in 250 ml vol. flask. Cool, dil. to mark with H_2O, and mix well. Pipet 50 ml into 150 ml beaker, add 1 ml of the K_2CrO_4 indicator, and titr. with the $AgNO_3$ soln until red color of Ag_2CrO_4 is permanent.

Acid-Soluble Calcium (*31*)—Official

2.084 *Method I.*

Weigh 2.5 g sample into 250 ml vol. flask, add 30 ml HNO_3 and 10 ml HCl, and boil 30 min. Cool, dil. to vol., mix, and filter if necessary. Transfer 25 ml aliquot to beaker and dil. to 100 ml. Add 2 drops bromophenol blue, **4.015(f)**. Add NH_4OH (1+4) until indicator changes from yellow to green (not blue). If overrun, bring back with HCl (1+4). (This gives pH of 3.5–4.0.) Dil. to 150 ml, bring to boil, and add 30 ml satd hot $(NH_4)_2C_2O_4$ soln slowly, stirring constantly. If color changes from green to blue or yellow again, adjust to green with the HCl. Digest on steam bath 1 hr, or let stand overnight, and cool to room temp. Filter supernatant thru quant. paper, gooch, or fritted glass filter, and wash ppt thoroly with NH_4OH (1+50). Place paper or crucible with ppt in original beaker and add mixt. of 125 ml H_2O and 5 ml H_2SO_4. Heat to 70° or above and titr. with 0.1N $KMnO_4$ until first slight pink appears. Correct for blank and calc. to Ca.

2.085 *Method II.*

Place CaC_2O_4 and filter paper from **2.086** in beaker in which pptn was made and add mixt. of 125 ml H_2O and 5 ml H_2SO_4. Heat to 70° or above and titr. with 0.1N $KMnO_4$ until first slight pink appears. Correct for blank and calc. to Ca.

Acid-Soluble Magnesium

2.086 *Gravimetric Method (32)—Official*

Weigh 2.5 g sample into 250 ml vol. flask, add 30 ml HNO_3 and 10 ml HCl, and boil 30 min. Cool., dil. to vol., and mix. Transfer to beaker aliquot of clear soln contg not >12 mg Mg, partially neutralize with NH_4OH, and add few drops Me red. Add NH_4OH until soln is yellow, then HCl until barely pink. Add 10 ml satd $(NH_4)_2C_2O_4$ soln for each 50 ml soln, adjust soln to pH 5.0 (faint pink) by addn of HCl (1+4) or NH_4OH (1+4), boil few min., cool, and again adjust to pH 5.0, adding more Me red if necessary. Stir thoroly and let soln stand until ppt settles.

Filter thru 11 cm paper fine enough to retain CaC_2O_4 and wash 10 times with hot H_2O. (Ppt may be used for Ca detn, **2.085**.) Evap. filtrate to ca 100 ml and add 5 ml *10% citric acid soln* and enough NH_4OH to make alk. to *bromothymol blue* indicator (0.1 g bromothymol blue dissolved in 1.6 ml 0.1N NaOH and dild to 25 ml with H_2O). Add 5 ml *10% $(NH_4)_2HPO_4$ soln.* Stir vigorously until ppt forms. Add 15 ml NH_4OH and let stand at least 2 hr, stirring frequently. If only small quantities of Mg are present and no ppt forms during stirring or after adding the 15 ml NH_4OH, let stand overnight.

Transfer ppt to small filter or filtering crucible. Wash with NH_4OH (1+9), and ignite slowly in crucible at temp. <900° (preferably in muffle with pyrometric control) until C is burned and then 1–2 hr at 950–1000°. Cool in desiccator and weigh as $Mg_2P_2O_7$.

Residue consists of $Mg_2P_2O_7$ and possibly $Mn_2P_2O_7$ and $Ca_3(PO_4)_2$. Correct for Mn as follows.

Dissolve residue in 10 ml H_2SO_4 (1+9), transfer to 250 ml erlenmeyer, and add 50 ml HNO_3 (1+3) and 2 ml H_3PO_4. Heat nearly to b.p., and add 0.3 g KIO_4 with swirling. Hold 30–60 min. at 90–100° or until color development is complete. Cool, and dil. to convenient vol. In another flask contg same quantities of reagents treated similarly, match color by adding std $KMnO_4$ soln, or compare with std $KMnO_4$ soln in colorimeter. From vol. $KMnO_4$ soln required, or reading of colorimeter, calc. wt $Mn_2P_2O_7$ in residue. Subtract this wt from total wt, and regard difference as $Mg_2P_2O_7$ which contains 21.84% Mg.

2.087 *Volumetric Method—Official*

Filter ppt of $MgNH_4PO_4$ from **2.086** thru asbestos pad on gooch. Remove excess NH_3 by washing with soln of equal vols alcohol and H_2O (6–10 washings). Transfer pad and ppt

quantitatively to beaker with H_2O (ca 50 ml). Add enough 0.1*N* H_2SO_4 from buret to dissolve ppt, and add small excess. Titr. excess acid with 0.1*N* NaOH, using *Me orange* or *mixed indicator* (0.02 g neutral red and 0.2 g bromocresol green dissolved in 100 ml alcohol) as indicator. 1 ml 0.1*N* acid =0.00122 g Mg.

If Mn is present, add 1 ml H_2SO_4 to soln from above titrn, and transfer to 200 ml vol. flask Dil. to vol., mix, and pipet 50 ml clear soln into beaker. Add 5 ml H_3PO_4, heat nearly to b.p., with stirring or swirling, add 0.3 g *KIO_4* for each 15 mg Mn, and hold 30–60 min. at 90–100° (*33*), or until color development is complete. Dil. to measured vol. contg not >20 ppm Mn and compare with $KMnO_4$ std in colorimeter.

Correct previous titrn, or calcd wt of Mg, for Mn present, taking account of dilns.

2.088 Water-Soluble Magnesium (*33*)—**Official**

(**a**) *In potassium-magnesium sulfate, magnesium sulfate, and kieserite.*—Weigh 1 g sample into 250 ml vol. flask, add 200 ml H_2O, and boil 30 min.; cool, dil. to vol. with H_2O, and mix. Transfer to beaker aliquot of clear soln contg <12 mg Mg. Dil. to ca 100 ml with H_2O and proceed as in (**b**), beginning "Add ca 1 g NH_4Cl . . . "

(**b**) *In other materials, including mixed fertilizers.*—Weigh 1 g sample into 500 ml vol. flask, add 350 ml H_2O, and boil 1 hr. Cool, dil. to mark, mix, and filter if necessary. Transfer to beaker aliquot contg <12 mg Mg, usually 200 ml. Add ca 1 g NH_4Cl for each 100 ml and few drops of Me red, and acidify with HCl. Proceed as in **2.086**, line 4, beginning "partially neutralize with NH_4OH . . . "

2.089 Water-Soluble Magnesium in Coarse Particles (*34*)—**Official**

Weigh 15 g sample of unground fertilizer, using spoon to convey the material. Transfer to 250 ml beaker, add 100 ml H_2O, cover with watch glass, and boil 30 min. Disintegrate lumps by rubbing with rubber bulb of medicine dropper. Pour thru No. 40 sieve, washing beaker and sieve with stream of tap H_2O thru rubber tubing attached to faucet (3″ sieve is most convenient.) Transfer residue on sieve to porcelain evapg dish, disintegrate lumps with the rubber bulb, and again wash on sieve. Repeat process until sepn is complete; 3 repetitions are usually sufficient. Do not force particles thru by rubbing on the screen. Wash final residue into 250 ml vol. flask with H_2O, let stand until clear, and decant as much H_2O as possible, keeping all mineral particles in flask. Det. Mg as in **2.086** or **2.087**, beginning "add 30 ml HNO_3 and 10 ml HCl, and boil 30 min." in **2.086**, and report as % Mg in original sample.

Acid-Soluble Manganese

2.090 *Colorimetric Method* (*35*)—*Official*

(Applicable to samples with not >5% Mn)

Place 1 g sample in 200 ml wide-neck vol. flask or 250 ml beaker. Add 10 ml H_2SO_4 and 30 ml HNO_3. Heat gently until brown fumes diminish; then boil 30 min. If org. matter is not destroyed, cool, add 5 ml HNO_3, and boil. Repeat process until no org. matter remains, and boil until white fumes appear. Cool slightly, and add 50 ml H_3PO_4 (1+9). Boil few min. Cool, dil. to 200 ml in vol. flask, mix, and let stand to allow pptn of $CaSO_4$. Pipet 50 ml clear soln into beaker. Heat nearly to b.p., with stirring or swirling, add 0.3 g *KIO_4* for each 15 mg Mn present, and hold 30–60 min. at 90–100°, or until color development is complete. Cool, and dil. to measured vol. that will provide satisfactory concn for colorimetric measurement by instrument chosen (usually <20 ppm Mn). Compare in colorimeter against std $KMnO_4$ soln, **22.072**, or in spectrophotometer at 530 mμ. Calc. to Mn.

Bismuthate Method (*36*)—*Official*

2.091 REAGENTS

(**a**) *Sodium bismuthate powder.*—80% $NaBiO_3$, contg not >0.0005% Mn, and not >0.002% Cl.

(**b**) *Potassium permanganate soln.*—0.0910*N*. Dissolve 2.876 g $KMnO_4$ in H_2O and dil. to 1 L. 1 ml = 1 mg Mn. Stdze as in **42.024.**

(**c**) *Ferrous sulfate soln.*—0.0910*N*. Dissolve 25.3 g $FeSO_4.7H_2O$, 25 ml H_2SO_4, and 25 ml H_3PO_4 in H_2O and dil. to 1 L. 1 ml = 1 mg Mn. Stdze with $KMnO_4$ near time of actual use. Place measured portion ca equiv. to max. quantity of Mn to be detd in erlenmeyer contg 200 ml cold H_2SO_4 (3+97), and titr. with the $KMnO_4$ soln.

2.092 DETERMINATION

To 1 g sample in erlenmeyer (preferably 300 ml), add 5–10 ml HNO_3 and 7 ml H_2SO_4. Evap. on hot plate to white fumes. Add few drops HNO_3, again evap. to white fumes, and repeat until org. matter is destroyed. Cool. Add 100 ml H_2O, 10 ml HNO_3, and just enough $NaBiO_3$ to give strong permanganate color, or if quantity of Mn is small, slight excess of $NaBiO_3$. Boil gently 2–3 min. If permanganate color or MnO_2 disappears, cool somewhat, and repeat bismuthate treatment. (Permanent permanganate color or persistence of MnO_2 indicates sufficient excess of bismuthate.) Add *satd $NaHSO_3$ soln* dropwise while stirring until Mn compounds are reduced and soln clears. Avoid large excess. Boil gently 2–3 min. Cool to room temp., and dil. to ca 100 ml. If soln contains <40 mg Mn, proceed with detn; if >40 mg Mn, transfer to 200 ml vol. flask, add 5 ml H_2SO_4 and

10 ml HNO_3, cool, dil. to vol., and mix. Pipet aliquot contg not >40 mg Mn into erlenmeyer and dil. to 100 ml with soln contg 5 ml H_2SO_4 and 10 ml HNO_3 in 100 ml.

Before continuing, prep. suction filters of asbestos washed with the H_2SO_4 and then with H_2O. (Glass filter tubes with perforated porcelain disks to support asbestos and connected with suction flask are satisfactory. Mn soln must not contact rubber.) Then complete detn without interruption. To the Mn soln at 20–30° add at least 0.25 g $NaBiO_3$ for each 10 mg Mn. Swirl contents of flask 1 min., add 100 ml H_2O, and mix. Filter with suction thru prepd filter and wash with cold H_2SO_4 (3+97) until washings show no pink tint. Disconnect suction flask, and add the $FeSO_4$ soln from buret until permanganate color disappears; then add at least 10% in excess with 1 ml as min. excess. Titr. excess $FeSO_4$ with the $KMnO_4$ soln to faint pink. From $KMnO_4$ equivalence of ml $FeSO_4$ soln used, subtract the $KMnO_4$ used in back-titrn. From difference calc. % Mn in sample.

Copper

Long Volumetric Method (37)—Official

2.093 REAGENTS

(**a**) *Sodium thiosulfate std soln.*—Dissolve 7.82 g $Na_2S_2O_3.5H_2O$ in H_2O and dil. to 1 L.

(**b**) *Copper nitrate std soln.*—Place 2.000 g pure electrolytic Cu in 1 L vol. flask, add 100 ml HNO_3, heat until Cu dissolves, and dil. with H_2O to vol. at room temp.

(**c**) *Potassium iodide soln.*—Dissolve 50 g KI in enough H_2O to make 100 ml.

(**d**) *Starch soln.*—Mix ca 1 g sol. starch with enough cold H_2O to make thin paste, add 100 ml boiling H_2O, and boil ca 1 min. while stirring.

(**e**) *Bromocresol green indicator.*—Dissolve 0.1 g tetrabromo-*m*-cresolsulfonphthalein in 1.5 ml 0.1*N* NaOH, and dil. to 100 ml with H_2O.

2.094 DETERMINATION

Weigh 2 g if sample contains <5% Cu; if >5%, weigh enough to furnish little under 0.1 g Cu. Place sample in 300 ml erlenmeyer, and add 5–10 ml HNO_3 and 7.0 ml H_2SO_4. Digest on hot plate to dense white fumes. If soln darkens owing to org. matter, cool somewhat, add little more HNO_3, and digest again to dense white fumes, repeating if necessary until org. matter appears to be destroyed. Cool, and add 25–30 ml H_2O. Boil 1 min., remove from hot plate, and stir occasionally ca 15 min. Filter into 250 ml erlenmeyer and wash filter and residue with 6 small portions of hot H_2O. Cool to room temp. and dil. to 100 ml.

Pass *H_2S* thru soln in erlenmeyer 10–15 min. Prep. wash soln by dilg 10 ml H_2SO_4 with enough H_2O to make 1 L and satg with H_2S. Filter sample soln thru paper of fine texture and wash paper and ppt with 7 small portions of the wash soln, keeping filter funnel covered with watch glass as much as possible. Reserve filtrate for Zn detn.

Place paper and ppt in glazed porcelain crucible and ignite at dull red heat until C is completely destroyed. Blow H_2S out of pptn flask and wash the CuS from H_2S delivery tube into flask with *Br-H_2O*. Add 5 ml HNO_3 to CuO in cold crucible and warm until CuO dissolves. (This may require 10 min., after which disregard insol. specks.) Wash soln into pptn flask with H_2O and dil. to 35 ml.

For stdzg the $Na_2S_2O_3$ soln, add, to another 250 ml erlenmeyer, aliquot of the std $Cu(NO_3)_2$ soln and more HNO_3 so that equiv. of 5 ml HNO_3 is present, and dil. to 35 ml. Hereafter treat all solns alike.

Add excesss of Br-H_2O and few glass beads. Boil until excess Br is entirely expelled and vol. is <30 ml. Cool slightly and add NH_4OH cautiously until mixt. is distinctly alk. Boil until odor of NH_3 is very faint. Add 5 ml HOAc and boil 1 min. more. Cool to room temp. and dil. to 25–30 ml. Add 2 ml of the KI soln and titr. with the $Na_2S_2O_3$ soln to light yellow. Add ca 1 ml of the cold starch soln and continue titrn to disappearance of starch-I color. Calc. Cu equivalence of the $Na_2S_2O_3$ soln from titrn of aliquot of the std $Cu(NO_3)_2$ soln, **2.093**(**b**), and from this factor calc. quantity of Cu in sample soln.

2.095 *Short Volumetric Method (38)—Official*

Place 2 g sample in 300 ml erlenmeyer and add 10 ml HNO_3 and 5 ml H_2SO_4. For stdzg the $Na_2S_2O_3$ soln, **2.093**(**a**), treat aliquot of the std $Cu(NO_3)_2$ soln, **2.093**(**b**), similarly. Digest on hot plate to white fumes. If soln darkens, owing to org. matter, cool slightly, add little more HNO_3, and digest again to white fumes, repeating operation if necessary until org. matter appears to be destroyed. Cool, add 50 ml H_2O, boil ca 1 min., and cool to room temp.

Add bromocresol green, **2.093**(**e**), then NH_4OH until indicator changes to light green (pH 4). Cool again to room temp., and if indicator changes back to more acid color, add NH_4OH dropwise until indicator becomes light green again, avoiding excess. Add 2 g *NH_4HF_2*, mix well, and let stand ca 5 min. Add 8–10 g KI, mix well, and titr. with the $Na_2S_2O_3$ soln to light yellow. Add ca 1 ml of the starch soln and continue titrg slowly until color is nearly same as just before addn of the KI and becomes no darker on standing 20 sec. Report as % Cu.

Zinc

2.096 *Gravimetric Method (39)—First Action*

(For samples contg 0.1% or more of Zn)

For samples contg <0.20% Zn, weigh 10 g or just enough to furnish 4 mg Zn; for samples contg 0.20% Zn or more, weigh 2 g. Treat samples as in **2.094** thru sepn of CuS. Evap. combined filtrate and washings to ca 100 ml. If soln is darker than light yellow or light green, add excess of satd $KMnO_4$ soln and heat to boiling, adding more $KMnO_4$ soln if necessary to maintain excess. Add *6% SO_2 soln* until Mn is reduced, then excess of 1–2 ml, and continue evapn to ca 80 ml. Cool, and add 5 ml *40% citric acid soln* and 2 drops bromophenol blue, **6.007(c)**. Add NH_4OH to slight change of indicator color, and cool to room temp. Adjust to pH 3.0 by adding NH_4OH or H_2SO_4 (1+1) dropwise. (For comparison, place 100 ml *0.05% citric acid soln*, pH 3.0, in another 250 ml erlenmeyer and add 2 drops of the bromophenol blue soln.)

Pass rapid stream of *H_2S* thru sample soln 45 min. Prep. wash soln contg 0.5 g citric acid/L and sat. with H_2S. Filter sample soln thru fine ashless paper. Use rubber policeman to loosen ppt sticking to flask and delivery tube and wash onto filter with jet of the wash soln. Wash paper and ppt 7 more times with small quantities of the wash soln, keeping funnel covered with watch glass as much as possible. Place paper and ppt in Pt crucible that has been ignited and weighed with cover. Ignite in uncovered crucible at low temp., preferably in muffle, until paper is oxidized, then 1 hr at 950–1000°. Place cover on crucible while hot, cool in desiccator contg H_2SO_4, and weigh as ZnO. Report as Zn.

2.097 *Colorimetric Method (38)—First Action*

(For samples contg <4% Zn)

To 2.5 g sample in Kjeldahl flask add ca 10 ml HNO_3 and exactly 10 ml H_2SO_4. Boil down to white fumes. If soln darkens owing to org. matter, add little more HNO_3 and boil down again to white fumes, repeating if necessary until org. matter is destroyed. Cool, and add 100 ml H_2O. Boil 3–5 min., and cool to room temp. Filter with suction thru mat of filter-paper pulp. Wash out flask, and wash filter at least 5 times with wash soln. Dil. filtrate to 250 ml in vol. flask. Dil. this soln to such vol. that 10 ml aliquot contains ca 20 mmg Zn. Pipet 10 ml aliquot into flask and titr. with $1N$ NH_4OH until neutral to Me red. Using another 10 ml aliquot, proceed as in **6.043**, adding same vol. of the NH_4OH after adding the 40 ml Soln A.

2.098 **Free Sulfur** (*40*)—**Official**

Ext. 1 g sample with *CS_2* in Soxhlet app., letting extn thimble drain at least 12 times. Transfer ext. to 250 ml beaker. Evap. the CS_2 in draft at room temp. Heat in oven 20 min. at 60–70°; then cool to room temp. Add 10 ml *satd soln of Br in CCl_4*, cover, and let stand ca 30 min., stirring several times. Add 15 ml HNO_3, cover, and let stand ca 30 min., stirring several times. Evap. on hot plate to ca 5 ml. Add 20 ml HCl and evap. to ca 5 ml. Add ca 50 ml H_2O, filter, and wash with 2% HCl. Add 2 drops bromophenol blue, **4.015(f)**, and then NH_4OH to first color change. Add HCl dropwise until distinctly acid, then 5 drops in excess; dil. to 150 ml, heat to boiling, and add 10% $BaCl_2.2H_2O$ soln slowly, dropwise until ca 50% excess is present. (1 ml 10% $BaCl_2.2H_2O$ soln ppts ca 0.013 g S.) Cover beaker and digest on steam bath at least 1 hr. Cool to room temp. and filter thru asbestos on gooch previously ignited at 500° and weighed. Wash 10 times with hot H_2O. Ignite in muffle at 500° at least 20 min. Cool in desiccator and weigh as $BaSO_4$. Calc. as S.

Acid-Forming or Nonacid-Forming Quality (*41*)—Official

2.099 REAGENTS

(**a**) *Mixed indicator.*—Weigh 0.1 g bromocresol green and 0.02 g Me orange into agate mortar, triturate, and slowly add 2 ml $0.1N$ NaOH. Dil. to 100 ml with H_2O.

(**b**) *Sodium carbonate-sucrose soln.*—Dissolve 106 g Na_2CO_3, or 286 g $Na_2CO_3.10H_2O$, and 50 g sucrose, in H_2O. Dil. to 1 L. Pipet 10 ml into 250 ml erlenmeyer, carefully add 30 ml $1N$ HCl, and boil gently few min. to remove CO_2. Titr. with $0.5N$ NaOH as directed below. Ml $0.5N$ NaOH used in titrg = soln blank.

2.100 DETERMINATION

If fertilizer mixt., ground as in **2.007**, contains <30% of sum [total N + available P_2O_5 + H_2O-sol. K_2O], weigh 1 g sample into 100 or 150 ml porcelain or Pyrex beaker. If sum of these percentages is 30 or more, use 0.5 g, and for salts of Na or K use 0.25 g. With pipet or buret add 10 ml of the Na_2CO_3-sucrose soln, and mix thoroly with sample, except for unmixed nitrate salts or for mixed fertilizers contg considerable nitrate N. For these, substitute 0.25 g carbon black for the sucrose. Place in sand bath to depth of mixt. in beaker and evap. to complete dryness.(To avoid loss by spattering, use cone of ashless filter paper folded so that base will just slip into beaker and touch

sides all around, with apex cut off to form vent ca 3 mm diam.) Place beakers in furnace heated to ca 250°, and raise temp. gradually to and hold at 575–600° (dull red) 1 hr. (It is not necessary that all C be removed.) Remove beaker and cool. Add 50 ml H_2O, cover with watch glass, and add 30 ml $1N$ HCl thru lip of beaker. After effervescence ceases, place covered beaker on hot plate or steam bath and hold just below b.p. 1 hr. Titr. by one of following methods:

(**a**) *With mixed indicator.*—Filter soln thru paper disk, or pad of asbestos that has been digested with $1N$ HCl and washed acid-free with H_2O, using gooch and suction. Wash with hot H_2O. To clear filtrate (ca 100 ml) add 0.4 ml of the mixed indicator, and titr. to light green (until green definitely predominates over yellow; pH 4.3). (Duplicate soln of fertilizer ash displaying max. acid color for this indicator may be used as comparison to det. first change. Titrn is conveniently carried out on white porcelain plate, with artificial daylight bulb placed at convenient angle above and back of plate.)

(**b**) *With glass electrode.*—Cool to room temp., and without filtering titr. soln in 150 ml beaker with $0.5N$ NaOH to pH 4.3, using glass electrode app., or other means of electrometric titrn, and continuous stirrer. Make usual blank titrn, using glass electrode.

Subtract algebraically ml $0.5N$ NaOH used in titrns from blank, **2.099**(**b**). For 1 g sample multiply result by 50; 0.5 g sample, by 100; 0.25 g sample, by 200. Positive values represent excess base in ash expressed as lbs $CaCO_3$/ton fertilizer. Negative values represent excess acidity in same terms.

% N found (**2.037**) ×35.7 is considered acid-forming power of the N in terms of equiv. lbs $CaCO_3$/ton fertilizer, and is given negative sign in calcg net acid-base balance.

% citrate-insol. P_2O_5 (**2.031**) ×28.2 = alky equiv. to 2 of the 3 Ca atoms of $Ca_3(PO_4)_2$, expressed as lbs $CaCO_3$/ton fertilizer. Correct net balance for fertilizer for this basicity, assumed to be relatively inactive in soil, by giving value negative sign.

Algebraic sum of acid-base balance of ash and corrections for N and citrate-insol. P_2O_5 is net balance of fertilizer expressed as lbs $CaCO_3$/ton. If negative, fertilizer is considered acid-forming; if positive, nonacid-forming.

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3. Caustic Poisons

PREPARATIONS CONTAINING PHENOL

Phenol

Method I. (1)—Official

(Applicable to commercial cresols, saponified cresol solns, coal tar dips and disinfectants, and to kerosene solns of phenols in absence of salicylates or β-naphthol.)

3.001 REAGENTS

(**a**) *Dilute nitric acid.*—Aërate HNO_3 until colorless and dil. 1 vol. of this acid with 4 vols H_2O.

(**b**) *Millon reagent.*—To 2 ml Hg in 200 ml erlenmeyer under hood, add 20 ml HNO_3. After first violent reaction, shake as needed to disperse Hg and maintain action. After ca 10 min., when action practically ceases even in presence of undissolved Hg, add 35 ml H_2O, and if basic salt seps, add enough of the dil. HNO_3 to dissolve it. Add 10% NaOH soln dropwise with thoro mixing until curdy ppt that forms after adding each drop no longer redissolves but disperses as permanent turbidity. Add 5 ml of the dil. HNO_3 and mix well. Prep. fresh daily.

(**c**) *Phenol std soln.*—Dissolve weighed quantity of pure phenol (congealing point not $<40°$) in enough H_2O to make not $<1\%$ soln. On day it is to be used, dil. to make 0.025% aq. soln (final std).

(**d**) *Formaldehyde soln.*—Dil. 2 ml 37% HCHO soln to 100 ml with H_2O.

(**e**) *Methyl orange indicator.*—0.5% aq. soln.

3.002 APPARATUS

(**a**) *Nessler cylinders.*—50 ml tall form, matched.

(**b**) *Test tubes.*—Approx. 180×20 mm, with rubber stoppers, marked at 25 ml.

(**c**) *Water bath for heating test tubes.*—Beaker contg disk of wire gauze raised ca 1″ from bottom may be used.

3.003 PREPARATION OF SAMPLE

(**a**) *Commercial cresol.*—Weigh by difference ca 2.5 g sample into 250 ml vol. flask, dissolve in 10 ml 10% NaOH soln, and dil. to mark with H_2O.

(**b**) *Saponified cresol solns, coal tar dips and disinfectants, kerosene solns of phenols, etc.*—Weigh by difference ca 5 g sample (or use 5 ml and calc. wt from density) into 250 ml vol. flask and dil. to mark with H_2O. With products consisting largely of kerosene, bring H_2O level to mark and take aliquots from aq. portion only.

3.004 DETERMINATION

Transfer 5 ml aliquot prepd soln to 200 ml vol. flask and promptly dil. to ca 50 ml. Add 1 drop Me orange, **3.001**(e), and then the dil. HNO_3 until soln is practically neutral; dil. to vol. and shake well.

Place 5 ml of the dild soln in each of 2 marked test tubes; in each of 2 addnl test tubes place 5 ml of the std phenol soln. Flow 5 ml of the Millon reagent down side of each tube, mix, and place tubes in boiling H_2O bath; continue boiling exactly 30 min., cool immediately and thoroly by immersion in bath of cold H_2O at least 10 min., and add 5 ml of the dil. HNO_3 to each tube.

Mix well and add 3 ml of the HCHO soln to one of each pair of tubes. Dil. all tubes to 25 ml mark with H_2O, stopper, shake well, and let stand overnight. (Tubes contg HCHO fade to yellow; others show orange or red tint.)

Pipet 20 ml from each of the 2 phenol tubes to 100 ml vol. flasks; add 5 ml of the dil. HNO_3 to each, dil. to mark, and mix. (Red flask contains "phenol std," yellow flask "phenol blank.") Transfer these solns to burets. Pipet 10 ml of each sample soln into Nessler tubes. (The orange or red constitutes the "unknown" and the yellow the "sample blank." Mark each Nessler tube distinctly to avoid confusion.) Add to "sample blank" tube measured quantity of "phenol std" and add same vol. of "phenol blank" to "unknown." Agitate thoroly (aided by insertion of rubber stoppers, if necessary), and compare colors. When tubes are brought to match, each ml phenol std used = 1% phenol if sample weighing exactly 5 g was used, or 2% if exactly 2.5 g was used.

NOTE.—Take following precautions: Pair of phenol tubes provides enough final solns to assay several unknowns, but all the latter must have accompanied phenol solns thruout entire process with identical reagents and treatment. If end point is inadvertently overrun it is possible to work back to it, but since mistakes may be made in this procedure it is better to repeat comparison on fresh portions from original tubes. Too much delay in matching tubes must be avoided after titrn is started, otherwise excess of HCHO present in blanks may have time after mixing to affect intensity of red color. Millon reagent is dangerously poisonous and should not be transferred with ordinary pipet and mouth suction unless protective trap is used.

3.005 *Method II.* (*2*)—*Official*

(Applicable to detn of phenol in presence of salicylates)

Weigh by difference 10 g sample into separator (or use 10 ml and calc. wt from density of sample). Add 50 ml kerosene and ext. with three 100 ml portions H_2O. Filter aq. exts thru wet filter into 500 ml vol. flask, dil. to vol. with H_2O, and proceed as in **3.004**.

When tubes are brought to match, each ml of the phenol std used = 1% phenol if sample weighing exactly 10 g was used.

SODA LYE

Carbonate and Hydroxide (*3*)—Official

3.006 DETERMINATION

Weigh ca 10 g sample from weighing bottle, dissolve in CO_2-free H_2O, and dil. to definite vol. Titr. aliquot of this soln with 0.5*N* HCl, **42.009–42.010**, using Me orange, **3.001**(e), and note total alky thus found. Transfer equal aliquot to vol. flask and add enough 10% $BaCl_2$ soln to ppt all carbonate, avoiding any unnecessary excess. Dil. to mark with CO_2-free H_2O, stopper, shake, and let stand. When liquid clears, pipet off one-half and titr. with the 0.5*N* HCl, using phthln; ml 0.5*N* acid required for this titrn $\times 2$ = ml 0.5*N* acid equiv. to NaOH present in original aliquot. Difference between this figure and ml 0.5*N* HCl required for total alky = ml 0.5*N* acid equiv. to Na_2CO_3 present in aliquot. Calc. % Na_2CO_3 and NaOH.

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4. Pesticides

GENERAL METHODS

4.001 Sampling—Procedure

No simple rules can be given for collection of sample representative of any particular lot of pesticides, as conditions may vary widely. Experienced judgment must be used in each instance. Examine shipping cases closely for code numbers, different labels, and other pertinent information. Give special attention to products subject to deterioration.

CAUTION: Use care in sampling and transporting toxic materials to avoid personal injury and contamination of transportation facilities in case of breakage. When dealing with rodenticides and weed-killers, avoid mutual contamination with other products during transportation.

(**a**) *Retail units.*—Generally 1 unit (1 lb if dry, 1 pint if liquid), preferably from unopened case or carton, is sufficient from each code or batch number. Size of sample is governed by composition of material and analytical methods to be used in examination.

(**b**) *Large-package dry products (25 lbs or more).*—Sample unopened containers when possible. Sample with trier long enough to reach bottom of container by inserting into container at one edge or corner and probing diagonally toward opposite edge or corner. Take subdivisions as follows: from 3 containers or less, take all; 3 to 10, take 3; >10, take 5.

Store samples in air-tight glass jars, tin containers, or cardboard canisters of at least 1 lb capacity.

(**c**) *Large-package liquid products (5 gallons or more).*—Agitate container before drawing sample. Invert emulsions, let stand, and then roll. Use glass, plastic tubing, or stainless steel trier with plunger, or rubber tubing for certain materials. Store samples in glass containers of at least 1 pint capacity with screw-top caps. Tin containers with screw-top caps may be used for some products. Take subdivisions as in (**b**).

4.002 Preparation of Sample—Official

Thoroly mix all samples before analysis. Det. H_2O-sol. As on samples as received, without further pulverization or drying. In case of lye, NaCN, or KCN, weigh large quantities in weighing bottles and analyze aliquots of the aq. solns.

4.003 Moisture—Official

(Applicable to Paris green, powd. Pb arsenate, Ca arsenate, Mg arsenate, Zn arsenite, powd. Bordeaux mixt., and Bordeaux mixt. with arsenicals)

Dry 2 g to constant wt at 105–110° and report loss in wt as moisture.

Total Arsenic—Official

Hydrazine Sulfate Distillation Method (1)

(Nitrates do not interfere in this method. Applicable to detn of total As in Paris green, Pb arsenate, Ca arsenate, Zn arsenite, Mg arsenate, and Bordeaux mixt. with arsenicals)

4.004 REAGENTS

(**a**) *Arsenious oxide std soln.*—0.1 or 0.05*N*. *See* **42.005–42.006.**

(**b**) *Iodine std soln.*—0.1 or 0.05*N*. *See* **42.016–42.017.**

(**c**) *Bromate std soln.*—0.1 or 0.05*N*. Dissolve ca 2.8 or 1.4 g $KBrO_3$ in boiled H_2O and dil. to 1 L. Stdze as follows: Pipet 25 ml aliquots of the As_2O_3 soln, (**a**), into 500 ml erlenmeyers. Add 15 ml HCl, dil. to 100 ml, heat to 90°, and titr. with the $KBrO_3$ soln, using 10 drops Me orange, (**g**). Do not add indicator until near end of titrn, and agitate soln continuously to avoid local excess of $KBrO_3$ soln. Add $KBrO_3$ soln very slowly near end point; at end point soln changes from red to colorless.

(**d**) *Hydrazine sulfate-sodium bromide soln.*—Dissolve 20 g $N_2H_4.H_2SO_4$ and 20 g NaBr in 1 L HCl (1+4).

(**e**) *Sodium hydroxide soln.*—Dissolve 400 g NaOH in H_2O and dil. to 1 L.

(**f**) *Starch indicator.*—Mix ca 2 g finely powd. potato starch with cold H_2O to thin paste; add ca 200 ml boiling H_2O, stirring constantly, and immediately discontinue heating. Add ca 1 ml Hg, shake, and let soln stand over the Hg.

(**g**) *Methyl orange indicator.*—0.05%. Dissolve 0.5 g Me orange in H_2O and dil. to 1 L.

4.005 APPARATUS (FIGURE 5)

Set 500 ml distn flask on metal gauze that fits over circular hole in heavy sheet of asbestos board, which in turn extends out far enough to protect sides of flask from direct flame of burner. First receiving flask holds 500 ml and contains 40 ml

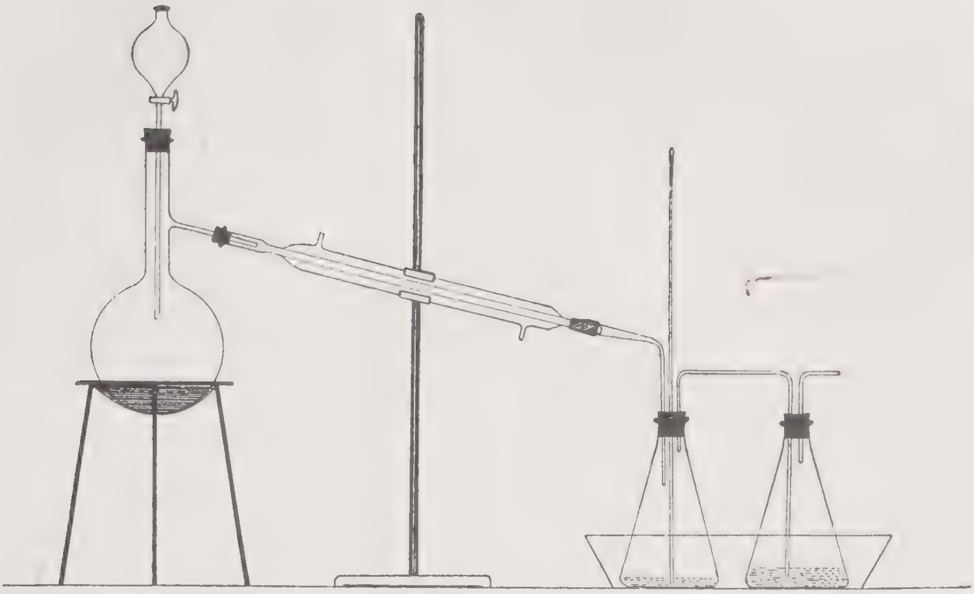

FIG. 5.—APPARATUS FOR DISTILLATION OF ARSENIOUS CHLORIDE

H_2O; second holds 500 ml and contains 100 ml H_2O. Vol. in first flask should not be >40 ml, otherwise compound of As may sep. that is difficult to dissolve without danger of loss of $AsCl_3$. Keep both flasks cool by placing them in pan thru which H_2O circulates, or that contains H_2O and ice.

4.006 DETERMINATION

Weigh quantity of sample contg not >0.4 g As and transfer to distg flask. Add 50 ml of the $N_2H_4.H_2SO_4$-NaBr soln, close flask with stopper that carries funnel tube, and connect side tube with condenser. Boil 2–3 min., add 100 ml HCl from dropping funnel, and distill until vol. in distg flask is reduced to ca 40 ml; add 50 ml more HCl and continue distn until contents of flask are again reduced to ca 40 ml. Wash down condenser, transfer contents of receiving flask to 1 L vol. flask, dil. to vol., mix thoroly, and proceed as in (**a**) or (**b**):

(**a**) Pipet 200 ml aliquot into erlenmeyer and nearly neutralize with the NaOH soln, using few drops of phthln, and keeping soln well cooled. If neutral point is passed, add HCl until again slightly acid. Neutralize with $NaHCO_3$, add 4–5 g excess, and add the std I soln from buret, shaking flask continuously until yellow color disappears slowly from soln. Add 5 ml of the starch indicator and keep adding the I soln dropwise until blue color is permanent.

(**b**) (*2*) Pipet 200 ml aliquot into erlenmeyer and titr. with the $KBrO_3$ soln, **4.004**(**c**), beginning "heat to 90° . . ."

Calc. % As. Report as As_2O_3 or As_2O_5, according to whether As is present in trivalent or pentavalent form. If condition of the arsenic is unknown, report as As.

Iodimetric Method (*3*)

(Applicable in presence of sulfides, sulfites, thiosulfates, and large quantities of S or org. matter)

4.007 REAGENT

Sodium thiosulfate soln.—Dissolve 13 g crystd $Na_2S_2O_3.5H_2O$ in H_2O and dil. to 1 L.

See **4.004** for other reagents and solns and **4.005** for app.

4.008 DETERMINATION

Weigh quantity of sample contg not >0.4 g As and transfer to distg flask. Add 50 ml $N_2H_4.H_2SO_4$-NaBr soln, **4.004**(**d**), and distill as in **4.006**. Make distillate to vol. in 1 L vol. flask, mix thoroly, and transfer 200 ml aliquot to 400 ml Pyrex beaker or porcelain casserole. Add 10 ml HNO_3 and 5 ml H_2SO_4, evap. to sirupy consistency on steam bath, and then heat on hot plate until white fumes of H_2SO_4 appear. Cool, and wash into 500 ml erlenmeyer. If vol. H_2SO_4 is appreciably lessened by fuming, add enough H_2SO_4 to make total vol. ca 5 ml. Dil. to 100–150 ml, add 1.5 g KI and boil until vol. is reduced to ca 40 ml. Cool soln under running H_2O, dil. to 100–150 ml, and add $Na_2S_2O_3$ soln, **4.007**, dropwise until I color just disappears. Nearly neutralize the H_2SO_4 with NaOH soln, **4.004**(**e**), finish neutralization with $NaHCO_3$, add 4–5 g excess, and titr. with std I soln as in **4.006**(**a**). From ml std soln used calc. % As in sample. Report as As_2O_3, As_2O_5, or As as in **4.006**.

Water-Soluble Arsenic—Official

(Applicable to detn of H_2O-sol. arsenic in Pb arsenate, Ca arsenate, Zn arsenite, Mg arsenate, and Bordeaux mixt. with arsenicals)

4.009 DETERMINATION

To 2 g original sample if powder, or 4 g if paste, in 1 L Florence flask, add 1 L recently boiled H_2O that has been cooled to 32°. Stopper flask and place in H_2O bath thermostated at 32°. Digest 24 hr, shaking hourly 8 hr during this period. Filter thru dry filter. If filtrate is not clear, refilter thru büchner contg paper and sufficient coating of Filter-Cel to give clear soln. Discard first 50 ml.

Transfer 250–500 ml *clear* filtrate to erlenmeyer, add 3 ml H_2SO_4, and evap. on hot plate. When vol. is ca 100 ml add 1 g KI, and continue boiling until vol. is ca 40 ml. Cool, dil. to ca 200 ml, and add $Na_2S_2O_3$ soln, **4.007,** dropwise, until I color is exactly removed. (Avoid use of starch indicator at this point.) Neutralize with $NaHCO_3$, add 4–5 g excess, titr. with the std I soln, shaking flask continuously, until yellow color disappears slowly, add 5 ml of the starch indicator, and continue titrn to permanent blue. Correct for quantity of std I soln necessary to produce same color, using same reagents and vol. From ml std I soln used calc. % H_2O-sol. As in sample.

4.010 Lead (*4*)—Official

(Applicable to such prepns as Bordeaux-Pb arsenate, Bordeaux-Zn arsenite, Bordeaux-Paris green, and Bordeaux-Ca arsenate)

Weigh 1 g powd. sample and transfer to beaker. Add 5 ml *HBr* (ca 1.38 sp. gr.) and 15 ml HCl, and evap. to dryness to remove As. Repeat treatment, add 20 ml more of the HCl and again evap. to dryness. Add 25 ml 2*N* HCl to residue, heat to boiling, filter immediately to remove SiO_2, and wash with boiling H_2O to vol. of 125 ml. See that all $PbCl_2$ is in soln before filtering; if it will not dissolve completely in 25 ml 2*N* acid, add 25 ml more, and dil. filtrate to 250 ml. Pass in H_2S until pptn is complete. Filter, and wash ppt thoroly with 0.5*N* HCl satd with H_2S. Save filtrate and washings for detn of Zn.

Transfer paper with the sulfides of Pb and Cu to 400 ml Pyrex beaker and completely oxidize all org. matter by heating on steam bath with 4 ml H_2SO_4 and ca 20 ml *fuming* HNO_3 in covered beaker. Evap. on steam bath and then completely remove HNO_3 by heating on hot plate until copious white fumes of H_2SO_4 appear. Cool, add 2–3 ml H_2O, and again heat to fuming. Cool, add 50 ml H_2O and 100 ml alcohol, and let stand several hr (preferably overnight). Filter thru gooch, previously washed first with H_2O, then with *acidified alcohol* (100 parts H_2O, 200 parts alcohol, and 3 parts H_2SO_4), and finally with alcohol, and dried at 200°. Wash ppt of $PbSO_4$ in crucible ca 10 times with the acidified alcohol, and then with alcohol, to remove H_2SO_4.

Dry at 200° to constant wt, keeping crucible covered to prevent loss from spattering. From wt $PbSO_4$ calc. % Pb in sample, using factor 0.6832.

Copper (*4*)—Official

(Applicable to such prepns as Bordeaux-Pb arsenate, Bordeaux-Zn arsenite, Bordeaux-Paris green, and Bordeaux-Ca arsenate)

4.011 *Electrolytic Method*

Evap. filtrate and washings from $PbSO_4$ pptn, **4.010,** to fuming; add few ml *fuming* HNO_3 to destroy org. matter, and continue evapn to ca 3 ml. Take up with ca 150 ml H_2O, add 5 ml HNO_3, and filter if necessary. Wash into 250 ml beaker, adjust vol. to 200 ml, and electrolyze, using rotating anode and weighed gauze cathode with current of 2–3 amperes. After all Cu has apparently deposited (ca 30 min.), add 15–20 ml H_2O to electrolyte and continue electrolysis few min. If no further deposition occurs on newly exposed surface of electrode, wash with H_2O without breaking current either by siphoning or quickly replacing beaker with electrolyte successively with 2 beakers of H_2O. Interrupt current, rinse cathode with alcohol, dry few moments in oven, and weigh. Calc. % Cu in sample.

4.012 *Volumetric Thiosulfate Method*

Proceed as in **4.011** to point at which filtrate and washings from $PbSO_4$ pptn are treated with fuming HNO_3 and evapd to vol. of ca 3 ml. Take up in ca 50 ml H_2O, add NH_4OH in excess, and boil to expel excess NH_3, as shown by color change in liquid and partial pptn. Add 3–4 ml HOAc (4+1), boil 1–2 min., cool, add 10 ml 30% KI soln, and titr. with std $Na_2S_2O_3$ soln, **29.041,** until brown color becomes faint. Add starch indicator, **4.004(f)**, and continue titrn cautiously until blue color due to free I entirely disappears. From ml std $Na_2S_2O_3$ soln used calc. % Cu in sample.

Zinc (*5*)—Official

(Applicable to such prepns as Bordeaux-Pb arsenate, Zn arsenite, Bordeaux-Zn arsenite, Bordeaux-Paris green, and Bordeaux-Ca arsenate)

4.013 REAGENT

Mercury-thiocyanate soln.—Dissolve 27 g $HgCl_2$ and 30 g NH_4SCN in H_2O and dil. to 1 L.

4.014 DETERMINATION

Conc. filtrate and washings from sulfide pptn, **4.010,** by gentle boiling to ca 50 ml, and continue evapn on steam bath to dryness. Dissolve residue in 100 ml H_2O contg 5 ml HCl, and add 35–40 ml

of the Hg-thiocyanate reagent with vigorous stirring. Let stand at least 1 hr with occasional stirring. Filter thru weighed gooch, wash with H_2O contg 20 ml Hg-thiocyanate reagent/L, and dry to constant wt at 105°. Calc. to % Zn, using factor 0.1312.

NOTE: Some Fe is usually present and during Zn detn should be in ferrous condition. In pptg sulfides pass H_2S into soln long enough to reduce Fe as well as to ppt Cu and Pb. $ZnHg(SCN)_4$ ppt normally is white, and occluded $Fe(SCN)_3$ should not give more than faint pink color.

Total Fluorine—Official

Lead Chlorofluoride Method (6)

4.015 REAGENTS

(**a**) *Fusion mixture.*—Mix 30 g anhyd. Na_2CO_3 with 40 g anhyd. K_2CO_3.

(**b**) *Lead chlorofluoride wash soln.*—Dissolve 10 g $Pb(NO_3)_2$ in 200 ml H_2O, dissolve 1 g NaF in 100 ml H_2O and add 2 ml HCl, and mix these 2 solns. Let ppt settle and decant. Wash ppt 4 or 5 times with 200 ml H_2O by decanting, and then add ca 1 L cold H_2O to ppt and let stand 1 hr or longer, with occasional stirring. Filter and use clear filtrate. (Prep. more wash soln as needed by adding more H_2O to ppt of PbClF and stirring.)

(**c**) *Silver nitrate std soln.*—0.1 or 0.2*N*. Stdze as in **42.029.**

(**d**) *Potassium or ammonium thiocyanate std soln.*—0.1*N*. Stdze by comparing with the std $AgNO_3$ soln under same conditions as in detn.

(**e**) *Ferric indicator.*—To cold satd Cl-free $FeNH_4(SO_4)_2.12H_2O$ soln add enough colorless HNO_3 to bleach brown color.

(**f**) *Bromophenol blue indicator.*—Grind 0.1 g of the powder with 1.5 ml 0.1*N* NaOH and dil. to 25 ml.

4.016 DETERMINATION

(**a**) *Samples difficult to decompose such as cryolite, and others that contain Al or appreciable quantities of siliceous material.*—Mix 0.5 g sample (or less if necessary to contain 0.01–0.10 g F) with 5 g fusion mixt. and 0.2–0.3 g *powd.* SiO_2, cover with 1 g fusion mixt., and heat to fusion over Bunsen burner. (Use of blast lamp is unnecessary since it is preferable not to heat much beyond melting temp. If much Al is present, uniform, clear, liquid melt cannot be obtained; particles of white solid will sep. in the liquid. Cooled melt should be colorless, or at least should not have more than gray color.)

Leach cooled melt with hot H_2O and when disintegration is complete filter into 400 ml beaker. Return insol. residue to Pt dish with jet of H_2O, add 1 g Na_2CO_3, dil. to 30–50 ml, and boil few min., disintegrating any lumps with flat-end rod. Filter thru same paper, wash thoroly with hot H_2O, and adjust vol. of filtrate and washings to ca 200 ml. Add 1 g *ZnO* dissolved in 20 ml HNO_3 (1+9), boil 2 min., stirring constantly, filter, and wash thoroly with hot H_2O. During this washing return gelatinous mass to beaker 3 times and thoroly disintegrate in wash soln, because proper washing of this ppt on filter is difficult. (Mass can easily be returned to beaker by rotating funnel above beaker while cutting ppt loose from paper with jet of wash soln.)

Add 2 drops of the bromophenol blue indicator to filtrate, and with cover glass almost entirely over beaker add HNO_3 (1+4) until color just changes to yellow. Make soln slightly alk. with 10% NaOH soln, and with cover glass on beaker boil gently to expel CO_2. Remove from burner; add the HNO_3 until color just changes to yellow and then the dil. NaOH until color just changes to blue; then add 3 ml 10% NaCl soln. (Vol. of soln at this point should be ca 250 ml.)

Add 2 ml HCl (1+1) and 5 g $Pb(NO_3)_2$ and heat on steam bath. As soon as the $Pb(NO_3)_2$ is in soln, add 5 g NaOAc, stir vigorously, and digest on steam bath 30 min. with occasional stirring. Let stand overnight, filter, and wash ppt, beaker, and paper once with cold H_2O, then 4 or 5 times with the PbClF soln, **4.015**(**b**), and then once more with cold H_2O.

Transfer ppt and paper to beaker in which pptn was made, stir paper to pulp, add 100 ml HNO_3 (5+95), and heat on steam bath until ppt dissolves. (5 min. is ample to dissolve ppt. If sample contains appreciable quantity of sulfates, ppt will contain $PbSO_4$, which will not dissolve. In such case heat 5–10 min. with stirring and consider PbClF to be dissolved.) Add slight excess 0.1*N* or 0.2*N* $AgNO_3$, digest on steam bath 30 min., and cool to room temp., protecting from light; filter, wash with cold H_2O, and det. $AgNO_3$ in filtrate by titrn with the std thiocyanate soln, using 10 ml of the ferric indicator. Subtract quantity of $AgNO_3$ found in filtrate from that originally added. Difference is amount required to combine with the Cl in the PbClF; from this difference calc. % F in sample on basis that 1 ml 0.1*N* $AgNO_3$ = 0.0019 g F.

(**b**) *Water-soluble fluorides in presence of organic matter.*—In presence of up to 50% org. matter such as flour, pyrethrum, tobacco powder, and derris or cubé powders, which readily decompose without addn of powd. SiO_2 and contain little or no sulfates, Al, or siliceous compounds, mix 0.5 g sample (or less if necessary to contain 0.01–0.1 g F) with 5 g fusion mixt., cover with 1 g fusion mixt., and heat to fusion over Bunsen burner. Leach cooled melt with hot H_2O, and when disintegration is complete, filter into 600 ml

beaker. Wash thoroly with hot H_2O and proceed as in (**a**), third par.

In presence of >50% org. matter or org. matter that is impractical to free without preliminary ashing, such as apple peel and pulp, transfer to Pt crucible enough sample to be representative of mixt. and to contain 0.01–0.1 g F. Add 15 ml H_2O and enough *F-free CaO* (0.3–0.4 g) to make mixt. distinctly alk. to phthln, mix with glass rod, and evap. to dryness on steam bath and in oven at 105°. Ignite at low heat, preferably in muffle (not >600°), until org. matter is thoroly charred. Pulverize, with glass rod, any lumps present in charred ash, mix with 5 g of the fusion mixt., and proceed as in (**a**), first par., beginning "cover with 1 g fusion mixt. . . . "

(**c**) *Water-soluble samples in absence of organic matter and appreciable quantities of sulfates or Al salts.*—In absence of org. matter or other interfering substances fusion may be omitted and detn made on aliquot of aq. soln contg 0.01–0.1 g F, as in (**a**), third par.

In presence of Al, as in samples contg Na_2SiF_6 and $KAl(SO_4)_2.12H_2O$, transfer sample to 400 ml beaker, dissolve in 150 ml hot H_2O, add 6 g of the fusion mixt., and boil. Add 1 g *ZnO* dissolved in 20 ml HNO_3 (1+9), boil 2 min. with constant stirring, filter into 500 ml vol. flask, and wash thoroly with hot H_2O. Cool to room temp. and dil. to vol. Transfer 200 ml aliquot contg 0.01–0.10 g F to 600 ml beaker and proceed as in (**a**), third par.

(**d**) *Sodium and Mg fluosilicates, or samples containing >5% sulfates in absence of Al and B, with or without moderate quantities of organic matter.*—With large quantities of Na_2SiF_6 and some other more volatile fluosilicates, *e.g.* $MgSiF_6$, where there is possibility of some F being evolved as SiF_4 before fusion is effected, or in samples contg appreciable quantities of sulfates, distill F as in **4.020**, and det. F in distillate as follows: Add several drops of the bromophenol blue indicator, make alk. with NaOH, and adjust vol. to ca 250 ml by gently boiling down vol. from 400 to 250 ml. Proceed as in (**a**), third par., beginning "Remove from burner; . . . "

NOTES.—These methods give accurate results for 0.01–0.10 g F. Below 0.01 g, results tend to be slightly low, and above 0.10 g, slightly high. Convenient sample to fuse is one contg 0.07–0.08 g F; too large sample may result in incomplete fusion. Large quantities of B compounds and alkali salts retard or prevent complete pptn of PbClF. B has greater effect when quantity of F is large than when it is small. In methods described B has little effect, and it may be disregarded in analysis of insecticides if quantity of F to be pptd is not >0.03 g. With some prepns contg $Na_2B_4O_7$ or H_3BO_3, where it is difficult to obtain representative mixt. when extremely small sample (0.1 g) is used for analysis, take larger sample and ppt PbClF from aliquot of fusion soln. Quantity of alkali carbonates specified in fusion and in washing of insol. residue is not large enough to cause low results. If sample contains S, remove it with CS_2 and det. F on air-dried residue, allowing in calcns for % S removed.

Modified Travers Method (7)

(Applicable in absence of B, Al, and large quantities of pyrethrum powder)

4.017 REAGENTS

(**a**) *Alcoholic potassium chloride soln.*—Dissolve 60 g KCl in 400 ml H_2O, add 400 ml alcohol, and test with phthln; if soln is not neutral, adjust to exact neutrality by adding NaOH or HCl soln.

(**b**) *Sodium hydroxide std soln.*—Approx. 0.2*N*. Prep. CO_3-free as in **42.031–42.032,** and stdze as in **42.033.**

4.018 DETERMINATION

Treat 0.5 g sample in small beaker with 20–25 ml H_2O. Add 0.3 g finely divided *pptd* SiO_2 and few drops Me orange. Add HCl dropwise until soln assumes apparently permanent pink; then add 2 ml excess, cover beaker with watch glass, and boil 1 min. Cool to room temp., add 4 g KCl, and stir until KCl dissolves. Add 25 ml alcohol and let stand 1 hr, stirring frequently. Filter thru gooch contg disk of filter paper covered with medium pad of asbestos. Wash ppt with the alc. KCl soln until one washing does not destroy color made by 1 drop 0.2*N* NaOH and phthln (usually 3–4 washings). Transfer crucible and contents to 400 ml beaker, add 100 ml recently boiled H_2O and 1–2 ml phthln, heat, and titr. with the std NaOH soln. Finish titrn with the F soln actively boiling. Calc. % F (1 ml 0.2*N* NaOH = 0.0057 g F).

Distillation Method (8)

(Applicable to H_2O-sol. or H_2O-insol. insecticides in absence of gelatinous SiO_2, B, and Al)

4.019 REAGENTS

(**a**) *Sodium alizarin sulfonate indicator.*—Dissolve 0.1 g Na alizarin sulfonate in 200 ml H_2O.

(**b**) *Thorium nitrate soln.*—Approx. 0.05*N*. Stdze in terms of g F/ml by titrg F obtained by distn from std NaF, as in **4.020.** In stdzg for use with **4.020**(**b**) add 5 ml satd $KMnO_4$ soln in addn to other reagents in distn flask.

4.020 DETERMINATION

(**a**) *In absence of organic matter.*—Weigh quantity of sample contg ca 0.09 g F, and with aid of little H_2O transfer to 250 ml Claisen distn flask contg 12 glass beads. Adjust to ca 30 ml and close flask with 2-hole rubber stopper, thru which pass thermometer and 4 mm glass tube, both of which extend into soln. (The 4 mm glass tube

extends ca 5 cm above rubber stopper and by means of rubber tube *E* connects still with 1 L Florence flask contg H_2O for steam generation. Flask is equipped with steam discharge, *H*, and pressure tube *G*. *See* Fig. 6.)

Bring H_2O in steam generating flask to b.p. with pinchcock, *F*, in release tube open. Connect distg flask to condenser, and add 25 ml H_2SO_4 thru top of 4 mm tube, using pipet or special funnel. With stopcock, *F*, open, connect rubber tubing to 4 mm tube. Light burner under Claisen flask. Regulate flow of steam by adjusting burner flames and stopcock, *F*, so that vol. of soln is held constant and temp. in flask *B* is kept at 145–150°. Continue distn until 400 ml distillate collects. Dil. to 500 ml in vol. flask., transfer 50 ml aliquot to tall-form 150 ml beaker, and add 5 drops indicator, **4.019(a)**. Adjust acidity with 1% NaOH soln and HCl (1+249) until pink color just disappears. Add 2 ml of the HCl, and titr. with 0.05*N* $Th(NO_3)_4$ to permanent pink, using buret graduated in 0.05 ml.

(**b**) *In presence of organic matter.*—In presence of moderate quantities of org. matter transfer sample contg ca 0.09 g F and contg not >0.2 g org. matter, with aid of little H_2O, to 250 ml Claisen distn flask contg 12 glass beads. Add 5 ml satd $KMnO_4$ soln, adjust to ca 30 ml, and proceed as in (**a**), beginning "close flask with 2-hole rubber stopper, . . . "

In presence of large quantities of org. matter, transfer sample to medium-size Pt dish, add 15 ml H_2O and enough *F-free CaO* to make mixt. distinctly alk. to phthln, mix with glass rod, and evap. to dryness on steam bath and in oven at 105°. Ignite at low heat, preferably in muffle (not >600°) until org. matter is thoroly charred. Pulverize any lumps present in charred ash with glass rod, transfer to 250 ml Claisen distn flask by brushing, and finally wash out dish with 30 ml 10% H_2SO_4. Except to add 22 ml instead of 25 ml H_2SO_4, proceed as in (**a**), par. 2.

NOTE: If coating of pptd SiO_2 forms on inside of distn flask, remove by treatment with hot concd alkali soln, as it is capable of retaining F during distn of some samples and giving it up, at least in part, in later distns.

Fluorine Present as Sodium Fluosilicate—Official

(B, CaO, and alum absent)

4.021 REAGENT

Alcoholic potassium chloride and sodium carbonate soln.—Dissolve 1.0 g Na_2CO_3 in 100 ml alc. KCl reagent, **4.017(a)**.

For other reagents *see* **4.017.**

4.022 DETERMINATION

Weigh 1 g sample into Pt dish, and add rapidly, with continuous stirring, 50 ml of the alc. KCl-Na_2CO_3 reagent. Do not let soln become acid, and if necessary use more reagent to insure alky. Continue stirring until all sol. portions of sample dissolve. Proceed as in **4.018,** beginning: "Filter thru

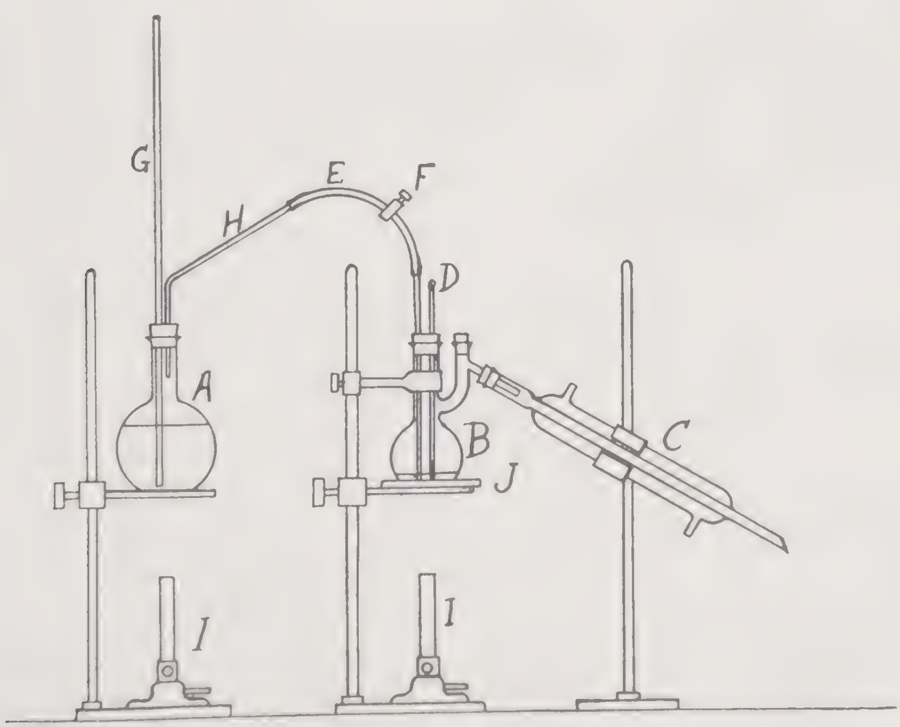

FIG. 6.—APPARATUS FOR DETERMINATION OF FLUORINE

gooch . . . " Calc. % Na_2SiF_6 (1 ml 0.2*N* NaOH =0.009403 g Na_2SiF_6).

PARIS GREEN

4.023 Moisture—Official—*See* **4.003**

4.024 Total Arsenic—Official—*See* **4.006**

Total Arsenious Oxide—Official

(Following methods det. only As present in trivalent form (As_2O_3) and Sb present in trivalent form (Sb_2O_3) in absence of ferrous and cuprous salts.)

Method I. (*9*)

4.025 REAGENT

Ammonium chloride soln.—Dissolve 250 g NH_4Cl in H_2O and dil. to 1 L.

For other reagents and solns *see* **4.004.**

4.026 DETERMINATION

Weigh 0.3 g sample and wash into erlenmeyer with 10–15 ml HCl (1+4) or 10–15 ml H_2SO_4 (1+4), followed by ca 100 ml H_2O, and heat on steam bath only long enough to complete soln, at temp. not >90°. (If H_2SO_4 is used, soln may be heated to boiling.) Cool, neutralize with $NaHCO_3$, add 4–5 g excess, and then add enough NH_4Cl soln to dissolve pptd Cu. Dil. somewhat and titr. with std I soln, **4.004(b)**, shaking flask continuously until yellow color disappears slowly from soln. Add 5 ml starch indicator, **4.004(f)**, and add I soln dropwise until permanent blue is obtained. Correct for quantity of I soln necessary to produce blue color with starch in presence of Cu (using equiv. wt of $CuSO_4$). From corrected ml std I soln used calc. % As_2O_3.

4.027 *Method II.* (*10*)

Weigh 1.5 g sample and wash into 250 ml vol. flask with 100 ml HCl (1+4), heating to max. of 90° if necessary to dissolve sample completely. Cool, and dil. to vol.

(**a**) Transfer 50 ml aliquot to 500 ml erlenmeyer, add 10 ml HCl, heat to 90°, and titr. with std $KBrO_3$ soln as in **4.004(c)**, beginning "titr. with the $KBrO_3$ soln, . . . " Or,

(**b**) Proceed as in (**a**) but make titrn without heating soln.

From ml $KBrO_3$ soln used calc. % As_2O_3.

4.028 Water-Soluble Arsenious Oxide—Official

To 1 g sample in 1 L Florence flask add 1 L recently boiled H_2O that has been cooled to 32°. Stopper flask and place in H_2O bath thermostated at 32°. Digest 24 hr, shaking hourly 8 hr during this period. Filter thru dry filter and transfer 250 ml filtrate to erlenmeyer; add 4–5 g $NaHCO_3$ and titr. with I soln, **4.004(b)**, to permanent blue, using starch indicator, **4.004(f)**. Correct for quantity of I soln necessary to produce same color, using same reagents and vol. Calc. quantity As_2O_3 present and express results as % H_2O-sol. As_2O_3.

Total Copper—Official

4.029 *Electrolytic Method*

Treat 2 g sample in beaker with 100 ml H_2O and ca 2 g NaOH and boil thoroly until all Cu ppts as Cu_2O. Filter, wash well with hot H_2O, dissolve ppt in hot HNO_3 (1+4), cool, transfer to 250 ml vol. flask, and dil. to mark. Transfer 50 or 100 ml aliquot to 250 ml beaker, adjust HNO_3 content to total of 12 ml, dil. to 200 ml, and electrolyze as in **4.011.** Calc. to % Cu.

4.030 *Volumetric Thiosulfate Method* (*11*)

Det. Cu in aliquot of the HNO_3 soln of Cu_2O, **4.029**, by titrg with std $Na_2S_2O_3$ soln as in **4.012,** and calc. % Cu.

LEAD ARSENATE

4.031 Moisture—Official

(**a**) *Powder.*—Dry 2 g to constant wt at 105–110° and report loss in wt as H_2O.

(**b**) *Paste.*—Proceed as in (**a**), using 50 g. Grind dry sample to fine powder, mix well, transfer small portion to sample bottle, and again dry 1–2 hr at 105–110°. Use this anhyd. material to det. total Pb and total As.

Total Arsenic—Official

4.032 *Method I.—See* **4.006**

4.033 *Method II.* (*12*)

(Not applicable in presence of Sb)

Dissolve 1 g powd. sample with HNO_3 (1+4) in porcelain casserole or evapg dish, add 5 ml H_2SO_4, and heat on hot plate to copious evolution of white fumes. Cool, add little H_2O, and again evap. until white fumes appear, to assure removal of last trace of HNO_3. Wash into 200 ml vol. flask with H_2O, cool, dil. to vol., and filter thru dry filter. Transfer 100 ml filtrate to erlenmeyer and proceed as in **4.009**, beginning "add 1 g KI, . . . " From ml std I soln used calc. % total As as As_2O_5.

4.034 Total Arsenious Oxide (*13*)—**Official**

Weigh 2 g powd. sample and transfer to 200 ml vol. flask, add 100 ml H_2SO_4 (1+6), and boil 30 min. Cool, dil. to vol., shake thoroly, and filter thru dry filter. Nearly neutralize 100 ml filtrate with NaOH soln, **4.004(e)**, using few drops phthln. If neutral point is passed, make acid again with the dil. H_2SO_4. Continue as in **4.009,** beginning

"Neutralize with $NaHCO_3$, . . ." From ml std I soln used calc. % As_2O_3.

Total Arsenic Oxide (*14*)—Official

4.035 REAGENTS

(a) *Potassium iodide soln.*—Dissolve 20 g KI in H_2O and dil. to 100 ml.

(b) *Thiosulfate std soln.*—Prep. ca 0.05*N* soln as follows: Dissolve 13 g $Na_2S_2O_3.5H_2O$ in recently boiled and cooled H_2O, filter, and dil. to 1 L with recently boiled and cooled H_2O. Stdze as follows:

Dissolve ca 0.7 g $PbHAsO_4$ in 50 ml HCl in erlenmeyer. If necessary to effect soln, heat on steam bath, keeping flask covered with watch glass to prevent evapn of acid. Cool to 20–25°, add 10 ml of the KI soln, (a), and 50 ml (or more if necessary to produce clear soln) NH_4Cl soln, **4.025**, and immediately titr. liberated I with the std $Na_2S_2O_3$ soln. When color becomes faint yellow, dil. with ca 150 ml H_2O and continue titrn carefully, dropwise, until colorless, using starch indicator, **4.004**(f), near end point. From wt $PbHAsO_4$ and ml $Na_2S_2O_3$ soln used, calc. titer of latter in terms of As_2O_5 (As_2O_5 in $PbHAsO_4$ =33.10%).

Prep. pure $PbHAsO_4$ by pouring soln of $Pb(NO_3)_2$ into soln of KH_2AsO_4, which should be in excess. Filter, dissolve ppt in smallest possible quantity boiling HNO_3 (1+4), and pour soln into 2 L H_2O contg 50 ml HNO_3. Filter, and dry ppt at 110°.

4.036 DETERMINATION

Weigh 0.5 g powd. sample and transfer to erlenmeyer. Add 25–30 ml HCl and evap. to dryness on steam bath. Add 50 ml HCl and proceed as in **4.035**(b), beginning "If necessary to effect soln, heat on steam bath, . . ." From ml std $Na_2S_2O_3$ soln used calc. % As_2O_5.

4.037 Water-Soluble Arsenic—Official

Proceed as in **4.009**, and calc. results as As_2O_5.

4.038 Total Lead (*15*)—Official

In 600 ml beaker on hot plate heat 0.5 g powd. sample and ca 25 ml HNO_3 (1+4). Filter to remove any insol. residue. Dil. to at least 400 ml, heat nearly to boiling, and add NH_4OH to slight pptn, then HNO_3 (1+9) to redissolve ppt, adding 1–2 ml excess. Pipet into this soln, kept almost boiling, 50 ml hot 10% K_2CrO_4 soln, stirring constantly. Decant while hot thru weighed gooch, previously heated to 140–150°, and wash several times by decanting and then on filter with boiling H_2O until washings are colorless. Dry $PbCrO_4$ at 140–150° to constant wt. From wt $PbCrO_4$ calc. % Pb, using factor 0.6411. ($PbCrO_4$ ppt may contain small quantity $PbHAsO_4$, which will cause slightly high results, but this error rarely is >0.1–0.2%.)

CALCIUM ARSENATE

4.039 Moisture—Official—*See* 4.003

4.040 Total Arsenic—Official—*See* 4.006

4.041 Total Arsenious Oxide (*16*)—Official

(a) *Not applicable in presence of nitrates.*—Weigh 1 g sample, transfer to 500 ml erlenmeyer, and dissolve in 100 ml HCl (1+3). Heat to 90° and titr. with std $KBrO_3$ soln, **4.004**(c), using 10 drops Me orange, **4.004**(g). From ml std $KBrO_3$ soln used calc. % As_2O_3.

(b) *Applicable in presence of small quantities of nitrates.*—Proceed as in (a) except to titr. at room temp.

4.042 Water-Soluble Arsenic—Official

Proceed as in **4.009**, and calc. results as As_2O_5. (In testing Ca arsenate by this method, low value for H_2O-sol. As is not assurance against plant injury when using this product.)

Total Calcium (*16*)—Official

Method I.

4.043 REAGENTS

(a) *Ammonium oxalate soln.*—Dissolve 40 g $(NH_4)_2C_2O_4.H_2O$ in 1 L H_2O.

(b) *Potassium permanganate std soln.*—0.1*N*. Prep. and stdze as in **42.023–42.024**.

4.044 DETERMINATION

Dissolve 2 g sample in 80 ml HOAc (1+3), transfer to 200 ml vol. flask, dil. to vol., and filter thru dry filter. Transfer 50 ml aliquot to beaker, dil. to ca 200 ml, heat to boiling, and ppt Ca with the $(NH_4)_2C_2O_4$ soln. Let beaker stand 3 hr on steam bath, filter soln, and wash ppt with hot H_2O. Dissolve ppt in 200 ml H_2O contg 25 ml H_2SO_4 (1+4), heat to ca 70°, and titr. with the $KMnO_4$ soln. From ml $KMnO_4$ soln used calc. % Ca.

4.045 *Method II.*

(Not applicable in presence of Pb)

Weigh 2 g sample, transfer to beaker, add 5 ml *HBr* (ca 1.38 sp. gr.) and 15 ml HCl, and evap. to dryness under hood to remove As. Repeat treatment, add 20 ml HCl, and again evap. to dryness. Take up with H_2O and little HCl, filter into 200 ml vol. flask, wash, and dil. to vol. Transfer 50 ml aliquot to beaker, add 10 ml HCl and few drops HNO_3, boil, and make slightly alk. with NH_4OH. Let stand few min. and filter. Dissolve ppt in HCl (1+4), reppt, filter thru same paper,

and wash with hot H_2O. To combined filtrates and washings add 20 ml HOAc (1+3) and adjust to ca 200 ml. Heat to boiling, ppt with $(NH_4)_2C_2O_4$ soln, **4.043(a)**, and let stand 3 hr on steam bath. Filter, and wash with hot H_2O. Ignite, and weigh as CaO; or, dissolve ppt in 200 ml H_2O contg 25 ml H_2SO_4 (1+4), heat to ca 70°, and titr. with $KMnO_4$ soln, **4.043(b)**. From wt CaO or ml $KMnO_4$ soln used calc. % Ca.

ZINC ARSENITE

4.046 Moisture—Official—*See* **4.003**

4.047 Total Arsenic—Official

Proceed as in **4.006** and calc. as As_2O_3.

Total Arsenious Oxide—Official

4.048 *Method I. (16)*

(**a**) Weigh 2 g sample and transfer to beaker. Dissolve in 80 ml HCl (1+4), wash into 200 ml vol. flask, and dil. to vol. Thoroly mix soln and filter thru dry filter. Transfer 25 ml aliquot to 500 ml erlenmeyer, add 20 ml HCl, and dil. to 100 ml. Heat to 90° and titr. with std $KBrO_3$ soln, **4.004(c)**. Or,

(**b**) Proceed as in (**a**) without heating soln.

4.049 *Method II.*

Proceed as in **4.026**, using appropriate Zn salt for blank detn.

4.050 Water-Soluble Arsenic—Official

Proceed as in **4.009**, and calc. results as As_2O_3.

4.051 Total Zinc *(16)*—**Official**

Transfer to beaker 25 ml aliquot of soln prepd for detn of total As_2O_3, **4.048**, and add 5 ml HCl. If much Fe is present, reduce by adding little *$NaHSO_3$* and heating on steam bath until odor of SO_2 practically disappears. Cool, dil. to ca 100 ml, and proceed as in **4.014**, beginning "add 35–40 ml of the Hg-thiocyanate reagent with vigorous stirring."

COPPER CARBONATE

Copper—Official

4.052 *Electrolytic Method*

Weigh 0.5 g sample, transfer to 150 ml Pt dish or 150 ml beaker, and dissolve in 25 ml HNO_3 (1+4). Dil. to ca 100 ml and det. Cu by electrolysis, as in **4.011**, beginning "electrolyze, using rotating anode and weighed gauze cathode . . ."

4.053 *Volumetric Thiosulfate Method*

Dissolve 0.25–0.5 g sample in 25 ml HNO_3 (1+4), dil. to ca 50 ml, and proceed as in **4.012**, beginning "add NH_4OH in excess, . . ."

BORDEAUX MIXTURE

4.054 Moisture—Official

(**a**) *Powder.*—Dry 2 g to constant wt at 105–110°. Report loss as H_2O.

(**b**) *Paste.*—Heat ca 100 g in oven at 90–100° until dry enough to powder readily and note loss in wt. Powder this partially dried sample and det. remaining H_2O in 2 g as in (**a**). Det. CO_2 as in **4.056**, both in original paste and in partially dried sample. Calc. total H_2O by following formula:

$$M = a + \frac{(100 - a)(b + c)}{100} - d,$$

in which $M = \%$ total H_2O in original paste; $a = \%$ loss in wt of original paste during first drying; $b = \%$ loss in wt of partially dried paste during second drying; $c = \%$ CO_2 remaining in partially dried paste after first drying; and $d = \%$ total CO_2 in original paste.

Carbon Dioxide *(17)*—**Official**

4.055 APPARATUS

Use 200 ml erlenmeyer with 2-hole stopper; in one hole fit dropping funnel with stem extending almost to bottom of flask, and thru other hole pass outlet of condenser that is inclined upward at 30° angle from horizontal. Connect upper end of condenser with $CaCl_2$ tube, which in turn connects with double U-tube filled in middle with pumice fragments, previously satd with *20% $CuSO_4.5H_2O$ soln* and subsequently dehydrated, and with $CaCl_2$ at either end. Connect 2 weighed U-tubes to absorb CO_2, first filled with porous soda-lime, and second, $\frac{1}{3}$ with soda-lime and $\frac{2}{3}$ with $CaCl_2$, placing the $CaCl_2$ at exit end of train. Attach Geissler bulb, partly filled with H_2SO_4, to last U-tube to show rate of gas flow, and connect aspirator with Geissler bulb to draw air thru app. Connect absorption tower filled with soda-lime to mouth of dropping funnel to remove CO_2 from air entering app.

4.056 DETERMINATION

Weigh into the erlenmeyer 2 g powder or 10 g paste and add ca 20 ml H_2O. Attach flask to app., omitting the 2 weighed U-tubes, and draw CO_2-free air thru app. until it displaces original air. Attach weighed U-tubes as in **4.055**, close stopcock of dropping funnel, pour into it 50 ml HCl (1+4), reconnect with soda-lime tower, and let acid flow into erlenmeyer, slowly if there is much CO_2, rapidly if there is little. When effervescence diminishes, place low Bunsen flame under flask and start flow of H_2O thru condenser, letting slow current of air flow thru app. at same time. Maintain steady but quiet boiling and slow air current thru app. Boil few min. after H_2O begins to con-

dense, remove flame, and continue air flow at ca 2 bubbles/sec. until app. is cool. Disconnect weighed absorption tubes, cool in balance case, and weigh. Increase in wt = CO_2.

Copper—Official

4.057 *Electrolytic Method*

(Also applicable to $CuCO_3$ and $CuSO_4$)

Dissolve powd. sample contg 0.2–0.25 g Cu in 45 ml HNO_3 (1+4). Filter if necessary, dil. to 200 ml, and electrolyze as in **4.011.**

4.058 *Volumetric Thiosulfate Method*

Dissolve 2 g powd. sample in ca 25 ml HNO_3 (1+4), dil. to 50 ml, add NH_4OH in excess, and heat. Without removing ppt that has formed, boil off excess NH_3, add 3–4 ml HOAc, cool, add 10 ml 30% KI soln, and titr. as in **4.012,** beginning "titr. with std $Na_2S_2O_3$ soln, . . . "

BORDEAUX MIXTURE WITH PARIS GREEN

4.059 Moisture—Official—*See* **4.054**

4.060 Carbon Dioxide—Official—*See* **4.056**

4.061 Total Arsenic—Official

Proceed as in **4.006,** using 2 g sample, and calc. results as As_2O_3.

4.062 Total Arsenious Oxide—Official

Proceed as in **4.026,** using 0.5–1.0 g sample.

4.063 Water-Soluble Arsenious Oxide—Official

Proceed as in **4.028,** using 2 g sample and slightly acidifying aliquot used with HCl (1+4) before adding excess $NaHCO_3$.

Copper—Official

4.064 *Electrolytic Method I.—See* **4.011**

4.065 *Electrolytic Method II.* *(Short Method)*

Dissolve 2 g powd. sample in 150 ml beaker with 5 ml HNO_3, add 25 ml *3%* H_2O_2 soln, and warm on steam bath 5–10 min. Add 25 ml more of the H_2O_2 soln, dil. to 100 ml, and electrolyze, using weighed gauze cathode, rotating paddle anode, and current of 2–3 amperes. After ca 20 min., add 15–20 ml more of the H_2O_2 soln. After all Cu deposits (which should require not >45 min.) and while current still flows, wash deposit with H_2O by siphoning. Interrupt current, rinse with alcohol, dry few min. in oven, weigh, and calc. % Cu. (Do not pass current >5–10 min. after all Cu deposits without adding more H_2O_2 soln.)

4.066 *Volumetric Thiosulfate Method* *See* **4.012**

BORDEAUX MIXTURE WITH LEAD ARSENATE

4.067 Moisture—Official—*See* **4.054**

4.068 Carbon Dioxide—Official—*See* **4.056**

4.069 Total Arsenic—Official

Proceed as in **4.006,** using 2 g sample, and calc. results as As_2O_5.

4.070 Water-Soluble Arsenic—Official

Proceed as in **4.009** and calc. results as As_2O_5.

Copper—Official

4.071 *Electrolytic Method—See* **4.011**

4.072 *Volumetric Thiosulfate Method—See* **4.012**

4.073 Lead—Official—*See* **4.010**

Lead and Copper—Official

Electrolytic Method (18)

4.074 APPARATUS

Electrodes.—Sandblasted cylindrical Pt gauze or plate; cathode, ca 50 mm high×25 mm diam.; anode, ca 50 mm high×50 mm diam.

4.075 DETERMINATION

Weigh 1 g powd. sample and transfer to 250 ml beaker. Add 15 ml HCl and 5 ml *HBr*, and evap. to dryness on steam bath. Repeat treatment, and finally, to remove last traces of As, add 20 ml HCl and again evap. to dryness. To residue add 25 ml H_2O and 15 ml HNO_3, and heat to boiling. Cautiously boil until most bromides and some chlorides are expelled (shown by changes in color, brown to green, then to blue). Evap. to dryness on steam bath. Add 10 ml H_2O and 15 ml HNO_3, and again evap. to dryness. Take up in 50 ml H_2O and 12 ml HNO_3 and heat until all salts are in soln. (It is unnecessary to filter off any siliceous material that may be present.) Dil. to 200 ml and electrolyze overnight, using current of 0.15 ampere and potential of 1.5–2 volts.

Add 15–20 ml H_2O to electrolyte and continue current few min. If there is no further deposition on newly exposed surfaces of electrodes, wash several times with H_2O without breaking current. Finally break current and wash once with MeOH or alcohol. Dry electrodes in oven 1 hr at 105–110°. Increase in wt cathode represents Cu present in sample, and increase in wt anode represents Pb as PbO_2. From increased wt cathode calc. % Cu. Because weighed PbO_2 is not completely anhyd.,

multiply by factor 0.8603 to obtain correct wt of Pb, and then calc. % Pb.

BORDEAUX MIXTURE WITH CALCIUM ARSENATE

4.076 Moisture—Official—*See* **4.054**

4.077 Carbon Dioxide—Official—*See* **4.056**

4.078 Total Arsenic—Official

Proceed as in **4.006,** using 2 g sample, and calc. results as As_2O_5.

4.079 Water-Soluble Arsenic—Official

Proceed as in **4.009** and calc. results as As_2O_5.

Copper—Official

4.080 *Electrolytic Method I.—See* **4.011**

4.081 *Electrolytic Method II.—See* **4.065**

4.082 *Volumetric Thiosulfate Method—See* **4.012**

SODIUM AND POTASSIUM CYANIDES

Cyanide (*19*)—Official

4.083 REAGENT

Silver nitrate soln.—0.1*N*. Stdze as in **42.027.**

4.084 DETERMINATION

Break sample into small lumps in mortar (do not grind). Weigh quickly ca 5 g in weighing bottle and wash into 500 ml vol. flask contg ca 200 ml H_2O. Add little $PbCO_3$ to ppt sulfides, if present, dil. to mark with H_2O, mix thoroly, and filter thru dry filter.

Transfer 50 ml aliquot to 400 ml beaker; add 200 ml H_2O, 5 ml NaOH soln (100 g/L H_2O), and 10 drops satd KI soln (or few crystals); and titr. to faint opalescence with the $AgNO_3$ soln. (In making this titrn, it is advantageous to have beaker over black surface.) From ml 0.1*N* $AgNO_3$ used calc. % CN. Reaction is represented by equation: $2NaCN + AgNO_3 = NaCN.AgCN + NaNO_3$ (1 ml 0.1*N* $AgNO_3$ = 0.005204 g CN).

Chloride (*20*)—Official

Method I.

4.085 REAGENT

Ammonium or potassium thiocyanate soln.—0.1*N*. Stdze by titrg against 0.1*N* $AgNO_3$, **4.083.**

4.086 DETERMINATION

Dil. 50 ml aliquot prepd soln, **4.084,** in beaker with equal vol. H_2O, add 1–2 ml *40% Cl-free HCHO* soln, stir well, and let stand 15 min. Acidify with 5 ml HNO_3 (1+1), add measured vol. 0.1*N* $AgNO_3$, **4.083,** enough to give excess, stir well, filter, wash, and titr. excess of Ag in combined filtrate and washings with the 0.1*N* thiocyanate soln, using the ferric indicator **4.015(e).** From ml 0.1*N* $AgNO_3$ minus ml thiocyanate soln used, calc. % Cl.

4.087 *Method II.*

Dil. 50 ml aliquot prepd soln, **4.084,** in distg flask to 100–150 ml with H_2O, acidify with slight excess of HOAc, and distill, passing vapors thru condenser, delivery end of which dips into 25 ml 10% NaOH soln, to absorb HCN. After distg all HCN (50 ml distillate), wash liquid remaining in distg flask into beaker, add 5 ml HNO_3 (1+1), and then measured vol. 0.1*N* $AgNO_3$, **4.083,** enough to give excess. Stir well, filter, wash, and titr. excess Ag in combined filtrate and washings with thiocyanate soln, **4.085,** using ferric indicator, **4.015(e).** From ml 0.1*N* $AgNO_3$ minus ml 0.1*N* thiocyanate soln used, calc. % Cl.

CALCIUM CYANIDE

Cyanide (*21*)—Official

4.088 REAGENT

Soda-lead soln.—Dissolve 20 g $Pb(OAc)_2$ in H_2O, dil. to 1 L, and add 200 g Cl-free Na_2CO_3.

4.089 DETERMINATION

Place ca 200 ml H_2O in 500 ml vol. flask and carefully dry neck of flask. Weigh ca 5 g sample in weighing bottle and transfer to flask with min. exposure to air. Wash mixt. down into flask and mix by swirling until soln is complete and the small quantity of CaC_2 has been decomposed. Add 25 ml of the soda-Pb soln, or enough to remove sulfides, close flask with rubber stopper, and shake thoroly, preferably 30 min. Dil. to mark, mix, and filter thru dry filter. Transfer 50 ml aliquot to 400 ml beaker and proceed as in **4.084,** beginning "add 200 ml H_2O, . . ." 1 ml 0.1*N* $AgNO_3$ = 0.005204 g CN. CN × 1.7702 = $Ca(CN)_2$.

Chloride (*21*)—Official

4.090 *Method I.*

Transfer 50 ml aliquot prepd soln, **4.089,** to beaker, and proceed as in **4.086.**

4.091 *Method II.* (*22*)

Transfer 50 ml aliquot prepd soln, **4.089,** to distg flask, and proceed as in **4.087.**

SOAP

Moisture (*22*)

4.092 *Toluene Distillation Method—Official*

Weigh ca 20 g sample into 300–500 ml flask; add 50 ml toluene (tech. grade is satisfactory);

and, to prevent foaming, add ca 10 g lump rosin (do not use powd.). Distill into Dean and Stark type distg tube receiver and continue distn until no more H_2O collects in receiver. Cool contents of tube to room temp., read vol. H_2O under toluene in tube, and calc. % H_2O.

4.093 Potassium and Sodium (*23*)—**Official**

Dissolve ca 5 g sample in H_2O, decompose with HCl (1+4), filter off H_2O, and wash fat with cold H_2O. Det. both K and Na in filtrate as in **6.015** and **6.020.**

MINERAL OILS

Unsulfonated Residue (*24*)—Official

4.094 REAGENT

Fuming 38N sulfuric acid.—In tared g-s. bottle (2.5 L acid bottle is convenient) mix fuming H_2SO_4 (free from N oxides) (A) with H_2SO_4 (B) to obtain mixed acid (C), contg slightly >82.38% total SO_3. Depending on strength of fuming acid available, use following proportions of 2 acids: 100 parts A (15–20% free SO_3) to 50 parts B; 100 parts A (20–30% free SO_3) to 75 parts B; and 100 parts A (50% free SO_3) to 140 parts B. Mix thoroly (considerable heat is generated), let cool, and again weigh to det. quantity mixed acid obtained. Det. exact strength of mixed acid (C) and also of reserve supply of acid (B) as follows:

Pour ca 50 ml into small beaker and fill ca 10 ml weighing bulb or pipet by slight suction, wiping off outside of bulb with moist, then with dry, cloth. Weigh on analytical balance and let acid flow slowly down sides of neck of 1 L vol. flask into ca 200 ml cold H_2O. (The sizes of bulb and flask give final soln ca 0.5*N*.) When bulb has drained, wash all traces of acid into flask taking precautions against loss of SO_3 fumes. Dil. to vol. and titr. from buret with std alkali, using same indicator as used in stdzg. Calc. SO_3 content of both acids, and add calcd quantity of reserve acid (B) to quantity of mixed acid (C) on hand to bring C to 82.38% total SO_3 (equiv. to 100.92% H_2SO_4). After adding required quantity of B, again analyze mixed acid to make certain it is of proper concn (±0.15% H_2SO_4). Keep acid in small bottles or in special dispenser bottle (*25*) to prevent absorption of H_2O from air.

4.095 DETERMINATION

Pipet 5 ml sample into 6" Babcock cream bottle, **15.071(a)**, either 9 g 50% or 18 g 30% type. To reduce viscosity of heavy oils, warm pipet after initial drainage by passing it several times thru flame; then drain thoroly. If greater accuracy is desired, weigh measured charge and calc. its exact vol. from wt and sp. gr. Slowly add 20 ml of the 38*N* H_2SO_4, gently shaking or rotating bottle and taking care that temp. does not rise above 60°. Cool in ice-H_2O if necessary. When mixt. no longer develops heat on shaking, agitate thoroly, place bottle in H_2O bath, and heat 10 min. at 60–65°, keeping contents of bottle thoroly mixed by shaking vigorously 20 sec. at 2 min. intervals. Remove bottle from bath and add H_2SO_4 until oil is in graduated neck. Centrifuge 5 min. (or longer if necessary to obtain constant vol. of oil) at 1200–1500 rpm. Read vol. of unsulfonated residue from graduations on neck of bottle and, to convert to ml, multiply reading from 9 g 50% bottle by 0.1 and reading from 18 g 30% bottle by 0.2. From result obtained calc. % by vol. unsulfonated residue.

MINERAL OIL-SOAP EMULSIONS

Water (*26*)

4.096 *Toluene Distillation Method—Official*

Weigh ca 25 g sample and proceed as in **4.092,** except use less rosin.

4.097 Total Oil (*27*)—**Official**

Weigh ca 10 g sample into Babcock cream bottle, **15.071(a)**. Dil. with ca 10 ml hot H_2O and add 5–10 ml H_2SO_4 (1+1). Heat in hot H_2O bath ca 5 min. to hasten sepn of oil, add enough satd NaCl soln to bring oil layer within graduated neck of bottle, whirl at 1200 rpm 5 min., and let cool. Read vol. of oil layer, det. density, and from these values calc. wt and %. From this % value deduct % fatty acids (and phenols if present), detd separately, to obtain % oil.

4.098 Soap (*26*)—**Official**

(Error will result if apparent molecular wt of fatty acids varies appreciably from that of oleic acid.)

Weigh 20 g sample into separator, add 60 ml petr. ether, and ext. mixt. once with 20 ml and 4 times with 10 ml 50% alcohol. Break emulsion if necessary by letting 1 or 2 ml 20% NaOH soln run down wall of separator. Then gently swirl separator and let stand few min. Drain alc. layers and wash successively thru petr. ether contained in 2 other separators. Combine alc. exts in beaker and evap. on steam bath to remove alcohol. Dissolve residue in ca 100 ml H_2O made alk. with NaOH. Transfer to separator, acidify with HCl or H_2SO_4, ext. 3 times with ether, and wash ether exts twice with H_2O. Combine ether exts, evap. in weighed beaker on steam bath, and weigh as fatty acids. From wt fatty acids calc. % soap in sample as Na or K oleate.

4.099 Unsulfonated Residue—Official

Using 5 ml of the recovered oil **4.097**, proceed as in **4.095**.

4.100 Ash *(28)*—**Official**

Evap. 10 g sample, or more if necessary, in Pt dish. Ignite, and leach charred mass with H_2O. Ignite residue, add leachings, evap. to dryness, ignite, and weigh. From this wt calc. % ash. Test ash for Cu, Ca, CaF_2, etc.

TOBACCO AND TOBACCO PRODUCTS

Nicotine

Silicotungstic Acid Method (29)—Official

(Includes nornicotine)

4.101 REAGENT

Silicotungstic acid soln.—Dissolve 120 g silicotungstic acid ($4H_2O.SiO_2.12WO_3.22H_2O$ or $SiO_2.12WO_3.26H_2O$) in H_2O and dil. to 1 L. (Acid should be white or pale yellow crystals, free from green color; soln should be free from cloudiness and green color. Of the several silicotungstic acids, $4H_2O.SiO_2.10WO_3.3H_2O$ and $4H_2O.SiO_2.12WO_3.20H_2O$ do not give cryst. ppts with nicotine and should not be used.)

4.102 DETERMINATION

Weigh quantity of prepn contg preferably 0.1–1.0 g nicotine. If sample contains very little nicotine (ca 0.1%), do not increase quantity to point where it interferes with distn. Wash with H_2O into 500 ml Kjeldahl flask, and if necessary add little paraffin to prevent frothing and few small pieces of pumice to prevent bumping. Add 10 g NaCl and 10 ml NaOH soln (30% by wt) and close flask with rubber stopper thru which passes stem of trap bulb and inlet tube for steam. Connect trap bulb to well-cooled condenser, lower end of which dips below surface of 10 ml HCl (1+4) in suitable receiving flask. Steam distill rapidly. When distn is well under way heat flask to reduce vol. of liquid as far as practicable without bumping or excessive sepn of insol. matter. Distill until few ml distillate shows no cloud or opalescence when treated with drop of the silicotungstic acid soln and drop HCl (1+4). Confirm alky of residue in distn flask with phthln.

Make distillate, which may amount to 1000–1500 ml, to convenient vol. (soln may be concd on steam bath without loss of nicotine), mix well, and pass thru dry filter if not clear. Test distillate with Me orange to confirm acidity. Pipet aliquot contg ca 0.1 g nicotine into beaker (if samples contain very small quantities of nicotine, aliquot contg as little as 0.01 g nicotine may be used). To each 100 ml of liquid add 3 ml HCl (1+4) and 1 ml silicotungstic acid for each 0.01 g nicotine supposed to be present. Stir thoroly and let stand overnight at room temp. Before filtering, stir ppt to see that it settles quickly and is in cryst. form. Filter on either ashless paper or gooch and wash with HCl (1+1000) at room temp. Continue washing for 2 or 3 fillings of filter after no more opalescence appears when few ml fresh filtrate is tested with few drops nicotine distillate. In case of paper, transfer paper and ppt to weighed Pt crucible, dry carefully, and ignite until all C is destroyed. Finally heat over Meker burner not >10 min. Wt residue×0.1141 = wt nicotine present in aliquot. In case of gooch, dry in oven 3 hr at 105° and weigh. Wt residue×0.1012 = wt nicotine present in aliquot.

DERRIS AND CUBÉ POWDER

Rotenone

Crystallization Method (30)—Official

4.103 REAGENTS

(**a**) *Purified rotenone.*—Dissolve rotenone in boiling CCl_4; cool in refrigerator or ice bath at 0–10° until pptn of rotenone-CCl_4 solvate stops. Filter thru büchner and wash once or twice with ice-cold CCl_4. Conc. filtrate, crystallize, and filter as before. Transfer cryst. residues to beaker, add ca twice their vol. alcohol and heat nearly to boiling. (Crystals need not dissolve completely.) Cool to room temp., filter thru büchner, and draw air thru cryst. residue until most of alcohol is removed. Remove rotenone from funnel, dry in air, and finally heat 1 hr at 105°. (Mother liquors may be concd and rotenone-CCl_4 solvate allowed to crystallize. Cryst. material may be used for further purification, or kept for prepn of wash solns or for seeding to induce crystn in analytical procedure.)

(**b**) *Rotenone-CCl_4 solvate.*—Ppt rotenone from CCl_4 soln, filter by suction, and dry in air.

(**c**) *Rotenone-CCl_4 wash soln.*—Sat. CCl_4 at 0°, and keep at 0° during use.

(**d**) *Alcohol saturated with rotenone at room temp.*

(**e**) *Charcoal, activated.*—Norit-A neutral, USP reagent or equiv.

4.104 PREPARATION OF SOLUTION

(**a**) Weigh 30 g (if sample contains >7% rotenone, use quantity to give 1.0–1.5 g rotenone in 200 ml aliquot) finely powd. root and 10 g of the C into 500 ml g-s. erlenmeyer. Add 300 ml $CHCl_3$, measured at known room temp.; fasten stopper securely and place flask on shaking machine. Agitate vigorously at least 4 hr, preferably interrupting shaking with overnight rest (or flask may be shaken continuously overnight). Filter mixt. rapidly into suitable flask, using fluted paper without suction and keeping funnel covered with watch glass to avoid loss from evapn. Stopper

flask and adjust temp. of filtrate to that of original $CHCl_3$.

(**b**) *Alternative extraction method.*—If sample is one in which ratio of rotenone to total ext. is >0.4, use quantity sufficient to contain 1.0–1.5 g rotenone and successively ext. 4 times with $CHCl_3$, using 300 ml $CHCl_3$ and 4 hr agitation for the first extn as in (**a**) and 200 ml and 2 hr each for second to fourth extns. Filter after each extn and return marc to flask for extn with fresh solvent. Finally combine exts, evap. almost to dryness, and use entire ext. to det. rotenone.

(**c**) *Extraction method for formulations containing 0.75–1.0% rotenone with or without sulfur and/or pyrethrins.*—Weigh two 50 g portions sample into sep. 500 ml g-s. erlenmeyers. Add 5 g of the C and 300 ml $CHCl_3$, measured at known room temp., to each. Stopper and continue as in (**a**).

4.105 DETERMINATION

Pipet 200 ml soln, **4.104** (or entire soln if alternative extn, (**b**), is used), into 500 ml Pyrex erlenmeyer and distill until ca 25 ml remains. (For formulations, **4.104**(**c**): In absence of S, combine the 2 exts in one of the erlenmeyers. In presence of S, remove all $CHCl_3$ on steam bath in air current, avoiding prolonged heating. Add 35 ml acetone to each residue and boil gently on steam bath to dissolve all resins. Remove from steam bath, stopper tightly, and hold 2 hr at 0–5°. Filter both acetone solns thru same 15 ml capacity, medium porosity, fritted glass büchner into single 500 ml erlenmeyer. Rinse and wash with acetone at 5°. Remove acetone as $CHCl_3$ was removed above.)

Evap. almost to dryness on steam bath in current of air. Remove remainder of solvent under reduced pressure, heating cautiously on steam bath when necessary to hasten evapn. (Suction may be applied directly to flask if stopper with vent is used to release pressure, so that excessive vac. may be avoided. Use flasks with slightly convex bottoms; do not use flasks below av. wt.) Dissolve ext. in 15 ml hot CCl_4 and again, in similar manner, remove all solvent. Repeat with another 10–15 ml portion hot CCl_4. (This treatment removes all $CHCl_3$ from resins. $CHCl_3$ ext. is usually completely sol. in CCl_4; if small quantities of insol. material are present, the purification described later will eliminate them.)

Dissolve residue in ca 10 ml hot CCl_4 and transfer to 50 ml erlenmeyer marked at 25 ml. Rinse the 500 ml flask with hot CCl_4 to remove last traces of ext., adding wash soln to CCl_4 soln in the 50 ml flask. Adjust vol. to 25 ml by evapg. on steam bath or by adding CCl_4. Cool flask in ice bath several min., stopper flask, and swirl until crystn is apparent. Seed with few crystals of rotenone-CCl_4 solvate if necessary to induce crystn. If at this stage only small quantity of cryst. material seps, add accurately weighed quantity of purified rotenone, **4.103**(**a**), estimated to be enough to assure that final result, expressed as pure rotenone, is at least 1 g. Then warm to dissolve completely, and again induce crystn. At same time prep. satd soln of rotenone in CCl_4, **4.103**(**c**), for washing. Place flasks contg ext. and washing soln in ice bath capable of holding temp. at 0°, and let stand overnight. (Store ice bath in refrigerator to keep ice from melting too rapidly.)

After 17–18 hr in ice bath, rapidly filter ext. thru weighed gooch fitted with filter paper disk, removing flask from ice bath only long enough to pour each fraction of ext. into crucible. Rinse cryst. residue from flask and wash under suction once with the ice-cold satd rotenone-CCl_4 wash soln. (Not >12–15 ml soln should be used for rinsing and washing.) Continue suction ca 5 min.; then dry to constant wt at 40° (ca 1 hr). Wt obtained is crude rotenone-CCl_4 solvate.

Break up contents of crucible with spatula, mix thoroly, and weigh 1 g into 50 ml erlenmeyer. Add 10 ml of the alcohol previously satd with rotenone at room temp., swirl flask few min., stopper tightly, and set aside at least 4 hr, preferably overnight, at same temp. Filter on weighed gooch fitted with filter paper disk. Rinse crystals from flask and wash under suction with alcohol satd with rotenone at temp. of recrystn (ca 10 ml usually required). Continue suction 3–5 min. and then dry crucible at 105° to constant wt (ca 1 hr).

Multiply wt residue, expressed in g, by wt total crude rotenone-CCl_4 solvate, and add 0.07 g to product as correction for rotenone held in soln in the 25 ml CCl_4 used in crystn. If any pure rotenone was added, subtract its wt from value obtained. This gives wt pure rotenone contained in aliquot of ext.

NOTE: Most important precaution in using this method is to keep temp. of CCl_4-rotenone wash soln and crucibles as near 0° as possible. Keep wash soln surrounded by crushed ice except when actually being used. In warm weather keep crucibles in refrigerator until ready to use.

4.106 Total Ether Extract—Official

Ext. 5 g finely powd. root with ether 48 hr in Soxhlet or other efficient extn app. Conc. ext. and filter off any insol. material present. Receive filtrate in tared beaker, evap. ether on steam bath, and dry in oven at 105° to constant wt.

PYRETHRINS

Mercury Reduction Method (31)

4.107 REAGENTS

(**a**) *Denigès reagent.*—Mix 5 g yellow HgO with 40 ml H_2O, and, while stirring, slowly add 20 ml H_2SO_4; then add addnl 40 ml H_2O and stir until all dissolves. Test for absence of mercurous Hg by

adding few drops of (**b**) to 10 ml and titrg with (**c**) as in **4.109**, beginning "Add 50 ml previously prepd and cooled dil. HCl . . ."

(**b**) *Iodine monochloride soln.*—Dissolve 10 g KI and 6.44 g KIO_3 in 75 ml H_2O in g-s. bottle; add 75 ml HCl and 5 ml $CHCl_3$, and adjust to faint I color (in $CHCl_3$) by adding dil. KI or KIO_3 soln. If much I is liberated, use stronger soln of KIO_3 than $0.01M$ at first, making final adjustment with $0.01M$ soln. Keep in dark and readjust when necessary.

(**c**) *Potassium iodate std soln.*—$0.01M$. Dissolve 2.14 g pure KIO_3, previously dried at 105°, in H_2O and dil. to 1 L. 1 ml of this soln = 0.0057 g pyrethrin I and needs no further stdzn.

(**d**) *Alcoholic sodium hydroxide soln.*—$1.0N$. Dissolve 40 g NaOH in alcohol and dil. to 1 L with alcohol.

(**e**) *Petroleum ether.*—Aromatic-free, b.p. 30–60°.

(**f**) *Ethyl ether.*—Peroxide-free, reagent grade.

4.108 PREPARATION OF SAMPLE

(**a**) *Pyrethrum powder.*—Ext. sample contg 40–150 mg total pyrethrins in Soxhlet or other efficient extn app. 7 hr with petr. ether. After extn is complete, evap. petr. ether to ca 40 ml, stopper flask, and place in refrigerator at $0 \pm 0.5°$ overnight. Filter cold ext. thru cotton plug satd with cold petr. ether, in stem of funnel, collecting filtrate in 250 ml erlenmeyer. Wash with three 15 ml portions cold petr. ether. Evap. filtrate and washings on H_2O bath, using air current, until <1 ml solvent remains.

Add 15–20 ml $0.5N$ alc. NaOH to flask contg pyrethrum ext., connect to reflux condenser, and boil gently 1–1.5 hr. Transfer to 600 ml beaker and add enough H_2O to bring vol. to 200 ml. Add few glass beads, or preferably use boiling tube, and boil down to 150 ml. Transfer to 250 ml vol. flask and add 1 g Filter-Cel and 10 ml 10% $BaCl_2$ soln. Do not shake before dilg to vol. Dil. to vol., mix thoroly, filter off 200 ml, neutralize with H_2SO_4 (1+4), using 1 drop phthln, and add 1 ml excess. (If necessary to hold soln overnight at this point, leave in alk. condition.)

(**b**) *Pyrethrum extracts in mineral oil.*—Weigh or measure sample contg 40–150 mg total pyrethrins, add 50 ml petr. ether and 1 g Filter-Cel, and place in refrigerator at $0 \pm 0.5°$ overnight. Filter thru gooch into 300 ml erlenmeyer and wash with three 15 ml portions cold petr. ether. Evap. filtrate and washings on H_2O bath, using air current, until <1 ml solvent remains.

Add 20 ml $1N$ alc. NaOH, or more if necessary, to flask contg pyrethrum ext., connect to reflux condenser, and boil gently 1–1.5 hr. Transfer to 600 ml beaker and add enough H_2O to make aq. layer 200 ml. If >20 ml alc. NaOH soln was used, add enough H_2O so that all alcohol is removed when vol. is reduced to 150 ml. Add few glass beads, or preferably use boiling tube, and boil aq. layer down to 150 ml. Transfer to 500 ml separator and drain aq. layer into 250 ml vol. flask. Wash oil layer once with H_2O and add wash H_2O to aq. portion. If slight emulsion still persists after draining aq. layer and washings, add 2–3 ml 10% $BaCl_2$ soln, but do not shake vigorously after adding the $BaCl_2$ because reversed emulsion difficult to sep. may form. To aq. soln in 250 ml flask add 1 g Filter-Cel and 10 ml or more of the $BaCl_2$ soln. Swirl gently and let stand 30 min. Dil. to vol., mix thoroly, and filter off 200 ml. Test filtrate with $BaCl_2$ soln to see if enough has been added to obtain clear soln. Neutralize with H_2SO_4 (1+4), using 1 drop phthln, and add 1 ml excess. (If necessary to hold soln overnight at this point, leave in alk. condition.)

4.109 DETERMINATION OF PYRETHRIN I—FIRST ACTION

Filter acid soln from **4.108**(**a**) or (**b**) thru 7 cm paper, coated lightly with suspension of Filter-Cel in H_2O, on büchner, and wash with three 15 ml portions H_2O. Transfer to 500 ml g-s. separator and ext. with two 50 ml portions petr. ether. Shake each extn at least 1 min., releasing pressure if necessary by inverting separator and carefully venting thru stopcock. Let layers sep. at least 5 min. or until aq. layer is clear before draining and re-extn. Reserve aq. layer for pyrethrin II detn. Do not combine petr. ether exts but wash each in sequence with same three 10 ml portions H_2O, and filter petr. ether exts thru small cotton plug into clean 250 ml separator. Wash separators and cotton in sequence with 5 ml petr. ether. Ext. the combined petr. ether solns with 5 ml $0.1N$ NaOH, shaking vigorously at least 1 min. Let layers sep. at least 5 min. before draining aq. layer into 100 ml beaker. Wash petr. ether with addnl 5 ml portion $0.1N$ NaOH and with 5 ml H_2O, adding washings to beaker. Add 10 ml Denigès reagent and let stand in complete darkness 1 hr at $25 \pm 2°$.

Add 20 ml alcohol and ppt HgCl with 3 ml satd NaCl soln. Warm to ca 60° and let stand several min. until ppt coagulates and settles. Filter thru small paper, transferring all ppt to paper, and wash with 10 ml or more hot alcohol. Wash with 2 or more 10 ml portions hot $CHCl_3$ and place paper and contents in 250 ml g-s. erlenmeyer. Add 50 ml previously prepd and cooled dil. HCl (3+2). Add 5 ml $CHCl_3$ or CCl_4 and 1 ml freshly adjusted ICl soln, and titr. with the KIO_3 soln, shaking vigorously at least 30 sec. after each addn, until no I color remains in $CHCl_3$ or CCl_4 layer. Take as end point when red color disap-

pears from solvent layer and does not return within 1–3 min. From ml std KIO_3 soln used in titrn and blank on Denigès reagent, calc. % pyrethrin I.

(KIO_3 reacts with mercurous Hg to form mercuric Hg and I; further addn of KIO_3 in presence of HCl oxidizes I to ICl:

$$2Hg_2Cl_2 + 4ICl = 4HgCl_2 + 2I_2$$

$$2I_2 + KIO_3 + 6HCl = KCl + 5ICl + 3H_2O$$

Addn of ICl does not change vol. relationship between mercurous Hg and KIO_3 soln, and aids in detg end point in titrn of small quantities of Hg.)

NOTE: Chrysanthemum monocarboxylic acid reacts with Denigès reagent to form series of colors beginning with phthln red, which gradually changes to purple, then to blue, and finally to bluish green. Color reaction is very distinct with 5 mg monocarboxylic acid, and quantities as low as 1 mg can usually be detected. Therefore no pyrethrin I should be reported if color reaction is negative.

With samples contg much perfume or other saponifiable ingredients, it may be necessary to use as much as 50 ml 1*N* alc. NaOH. When lethanes are present, after washing HgCl ppt with alcohol and $CHCl_3$, wash once more with alcohol and then several times with hot H_2O.

4.110 DETERMINATION OF PYRETHRIN II (*32*)—OFFICIAL

If necessary, filter aq. residue from petr. ether extn thru gooch. Conc. filtrate to ca 50 ml and transfer to 500 ml g-s. separator. Wash beaker with three 15 ml portions H_2O. Acidify with 10 ml HCl and sat. with NaCl. (Acidified aq. layer must contain visible NaCl crystals thruout following extns.)

Ext. with 50 ml ether, drain aq. layer into second separator, and ext. again with 50 ml ether. Continue extg and draining aq. layer, using 35 ml for third and fourth extns. Shake each extn at least 1 min., releasing pressure, if necessary, by inverting separator and carefully venting thru stopcock. Let layers sep. at least 5 min. or until aq. layer is clear before subsequent draining and extn. Combine ether exts, drain, and wash with three 10 ml portions satd NaCl soln. Filter ether exts thru cotton plug into 500 ml erlenmeyer and wash separator and cotton with addnl 10 ml ether. Evap. ether on H_2O bath and remove any fumes of HCl with air current and continued heating not >5 min. Dry 10 min. at 100°.

Add 2 ml neutral alcohol and 20 ml H_2O and heat to dissolve acid. Cool, filter thru gooch if necessary, add 1 or 2 drops phthln, and titr. with 0.02*N* NaOH (1 ml = 0.00374 g pyrethrin II). Check normality of 0.02*N* NaOH on same day sample is titrd.

FORMALDEHYDE

Formaldehyde in Solutions

Hydrogen Peroxide Method (33)—Official

4.111 REAGENTS

(**a**) *Sulfuric acid.*—1*N*. Prep. and stdze as in **42.037–42.038**.

(**b**) *Sodium hydroxide soln.*—1*N*. Stdze against (**a**), using litmus or bromothymol blue indicator. 1 ml = 30.03 mg HCHO.

(**c**) *Hydrogen peroxide soln.*—Commercial, contg ca 3% H_2O_2. If acid, neutralize with NaOH, (**b**), using litmus or bromothymol blue indicator.

(**d**) *Litmus indicator.*—Soln of purified litmus of such concn that 3 drops gives distinct blue color to 50 ml H_2O.

(**e**) *Bromothymol blue indicator.*—Dissolve 1 g bromothymol blue in 500 ml alcohol, 50% by vol.

4.112 DETERMINATION

Pipet 50 ml of the NaOH soln into 500 ml erlenmeyer and add 50 ml of the H_2O_2. Add weighed quantity sample (ca 3 g) from weighing pipet, letting point of pipet reach nearly to liquid in flask. Place funnel in neck of flask and heat on steam bath 5 min., shaking occasionally. Remove from bath, wash funnel with H_2O, cool flask to room temp., and titr. excess NaOH with the 1*N* acid, using bromothymol blue or litmus. (It is necessary to cool flask before titrn to obtain sharp end point with litmus.) From ml 1*N* NaOH used and wt sample, calc. % HCHO according to following equation:

$$NaOH + HCHO + H_2O_2 = HCOONa + 2H_2O.$$

If the HCHO soln contains appreciable free acid, titr. separate portion and calc. acidity as % HCOOH. Correct for this acidity in calcg % HCHO.

4.113 *Cyanide Method (34)—Official*

(Applicable only to dil. solns)

Treat 15 ml 0.1*N* $AgNO_3$, **4.083**, with 6 drops HNO_3 (1+1) in 50 ml vol. flask, add 10 ml *KCN soln* (3.1 g in 500 ml H_2O), dil. to mark, shake well, filter thru dry filter, and titr. 25 ml filtrate with 0.1*N* NH_4SCN, **4.085**, as in **6.068**. Acidify another 15 ml portion 0.1*N* $AgNO_3$ with 6 drops of the dil. HNO_3 and treat with 10 ml of the KCN soln to which has been added measured quantity of sample (wt calcd from sp. gr.) contg not >25 mg HCHO. Dil. to 50 ml, filter, and titr. 25 ml aliquot with the 0.1*N* NH_4SCN for excess of Ag as before. Difference between ml NH_4SCN used in these 2 titrns × 2 = ml 0.1*N* NH_4SCN corresponding to KCN used by the HCHO. Calc. %

HCHO present. 1 ml 0.1*N* NH_4SCN = 3.00 mg HCHO.

4.114 Formaldehyde in Seed Disinfectants (*35*)—**Official**

(Applicable to detn of HCHO absorbed in inert carrier, such as Bentonite, talc, charcoal, sawdust, etc.)

Weigh ca 5 g sample contg 0.3–0.5 g HCHO in weighing bottle and transfer to 800 ml Kjeldahl flask. Add 25 ml H_2O and 12 ml H_2SO_4 (1+4). Steam distill rapidly, passing vapors thru condenser with delivery end dipping into 25 ml H_2O in 500 ml vol. flask. Collect ca 450 ml distillate, keeping vol. in distg flask nearly constant with aid of small flame. After distn, wash delivery tube, and dil. distillate to vol. with H_2O.

Into each of two 200 ml vol. flasks measure 20 ml 0.1*N* $AgNO_3$. To each flask add 12 drops HNO_3 (1+1) and 30 ml H_2O. To one of flasks add slowly, with constant shaking, 30 ml *KCN soln* (3.1 g in 1000 ml H_2O). Dil. to vol., shake well, and filter thru dry filter. To 100 ml filtrate add 3 ml HNO_3 and 5 ml $FeNH_4(SO_4)_2$ indicator, **4.015(e)**, and titr. with 0.1*N* KCNS.

Pipet 25 ml of the HCHO distillate into small beaker contg 30 ml of the KCN soln, mix well, and add slowly, with constant shaking, to second flask contg the acidified $AgNO_3$ soln. Dil. to vol. with H_2O, filter, acidify 100 ml filtrate with 3 ml HNO_3, and titr. with the KCNS soln, using the $FeNH_4(SO_4)_2$ indicator.

Difference between ml KCNS soln used in these 2 titrns×2 = ml 0.1*N* KCNS equiv. to the HCHO. Calc. % HCHO present. 1 ml 0.1*N* KCNS = 3.00 mg HCHO.

LIME SULFUR SOLUTIONS AND DRY LIME SULFUR

Soluble Sulfur (*36*)—Official

(Use low S reagents)

4.115 PREPARATION OF SAMPLE

(**a**) *Solutions.*—Accurately weigh ca 10 g soln, transfer to 250 ml vol. flask, and immediately dil. to mark with recently boiled and cooled H_2O. Mix thoroly and either take necessary aliquots in individual pipets in least possible time for detns or transfer to small bottles, filling them completely and avoiding contact of soln with air as much as possible. Stopper bottles, seal with paraffin, and store in dark, cool place.

(**b**) *Dry lime-sulfur.*—Stir thoroly 5 g sample with ca 50 ml H_2O in 250 ml beaker. Let settle and decant thru filter paper into 250 ml vol. flask. Repeat extn with H_2O until filtrate is colorless and ca 200 ml is obtained. Transfer residue to paper, wash with hot H_2O, cool to room temp., and dil. to vol. Dry residue 1.5 hr at 105°, and reserve for free S and sulfite S detns in residue, if desired. (Ext. S from dry residue with CS_2, evap. on steam bath or in air current, dry 15 min. at 105°, weigh, and calc. % S.)

Prep. soln in least possible time and keep beaker and funnel covered as much as possible.

4.116 DETERMINATION

Transfer with clean, dry pipet 10 ml prepd soln, **4.115(a)** or (**b**), to 250 ml beaker. Partially cover with cover glass and add 2–3 g *Na_2O_2* in small portions, with stirring, from tip of spatula. Continue addn of Na_2O_2 until all S appears to be oxidized to sulfate (yellow color disappears). Add slight excess of Na_2O_2, completely cover beaker with cover glass, and heat on steam bath, stirring occasionally, 15–20 min.

Wash off cover glass and sides of beaker, acidify with HCl (1+4), and filter if necessary. Dil. to 150–200 ml, heat to boiling, add 10% $BaCl_2$ soln (11 ml/1 g $BaSO_4$), with constant stirring, at such rate that ca 4 min. is required to add necessary quantity. Let stand until clear and cool, filter thru quant. paper, wash until Cl-free, ignite carefully, and heat to constant wt over Bunsen burner. Calc. % S from wt $BaSO_4$, using factor 0.1374.

Thiosulfate Sulfur (*36*)—Official

4.117 REAGENT

Ammoniacal zinc chloride soln.—Dissolve 50 g $ZnCl_2$ in ca 500 ml H_2O, add 125 ml NH_4OH and 50 g NH_4Cl, and dil. to 1 L.

4.118 DETERMINATION

To 50 ml H_2O in 200 ml vol. flask add 50 ml soln prepd as in **4.115(a)** or (**b**). Add slight excess of the ammoniacal $ZnCl_2$ soln and dil. to mark. Complete detn as rapidly as possible. Shake thoroly and filter thru dry filter. To 100 ml filtrate add few drops Me orange or Me red, **4.004(g)** or **2.034(i)**, and exactly neutralize with 0.1*N* HCl. Titr. neutral soln with 0.05*N* I, **4.004(b)**, using few drops starch indicator, **4.004(f)**. From ml I soln used calc. % thiosulfate S present. (Factor of I soln in terms of As_2O_3×1.296 = equiv. in thiosulfate S.)

Sulfide Sulfur

4.119 *Zinc Chloride Method* (*36*)—*Official*

To 10–15 ml H_2O in small beaker add 10 ml aliquot soln prepd as in **4.115(a)** or (**b**). Calc. quantity of ammoniacal $ZnCl_2$ soln, **4.117**, necessary to ppt all S in aliquot and add slight excess. Stir thoroly, filter, wash ppt twice with cold H_2O, and transfer paper and ppt to beaker in which pptn was made. Cover with H_2O, disintegrate

paper with glass rod, and add ca 3 g *Na_2O_2*, keeping beaker well covered with watch glass. Warm on steam bath with frequent shaking until all S is oxidized to sulfate, adding more Na_2O_2 if necessary. Acidify slightly with HCl (1+4), filter to remove shreds of paper, wash thoroly with hot H_2O, and det. S in filtrate as in **4.116.**

4.120 *Indirect Method—Official*

Difference between soluble S and sum of thiosulfate S and sulfate S = sulfide S.

4.121 Sulfate Sulfur—Official

Slightly acidify soln from **4.118** with HCl (1+4) and heat to boiling. Add slowly, with constant stirring, slight excess 10% $BaCl_2$ soln, boil 30 min., let stand overnight, and filter. Calc. S from wt $BaSO_4$, and report as % sulfate S.

4.122 Total Calcium *(36)*—**Official**

To 25 ml prepd soln, **4.115(a)** or **(b)**, add 10 ml HCl, evap. to dryness on steam bath, treat with H_2O and few ml HCl (1+4), warm until all $CaCl_2$ dissolves, and filter to remove S and any SiO_2 present. Dil. filtrate to 200–250 ml, heat to boiling, add few ml NH_4OH in excess, and then add excess satd $(NH_4)_2C_2O_4$ soln. Continue boiling until pptd CaC_2O_4 assumes well defined granular form, let stand 1 hr, filter, and wash few times with hot H_2O. Ignite in Pt crucible over blast lamp to constant wt and calc. % Ca.

ANT POISONS AND RODENTICIDES

4.123 Alpha-Naphthylthiourea *(37)*—**First Action**

(a) *In technical grade chemical.*—Transfer 0.2 g sample to Kjeldahl flask and proceed as in **2.036.** % N × 7.220 = % α-naphthylthiourea.

(b) *In fat-free mixtures.*—Weigh sample equiv. to ca 0.2 g α-naphthylthiourea and transfer to Soxhlet extn thimble. Mix and cover with thin padding of asbestos. Ext. with acetone overnight, or 4–5 hr on each of 2 days. Transfer acetone ext. to Kjeldahl flask, place on steam bath, and with aid of air current, evap. most of acetone. Expel last few ml with air current only. Proceed as in **(a)**.

(For samples contg only siliceous material as filler, acetone extn may be omitted unless large sample is required, which would cause serious bumping. Ext. samples contg <10% α-naphthylthiourea.)

(c) *In mixtures containing greasy material.*—If much greasy material is present, as in some prepd bait, use sample equiv. to ca 0.2 g α-naphthylthiourea, ext. sample in Soxhlet app. with petr. ether 1 hr, withdraw and discard petr. ether, ext. with acetone, and proceed as in **(b)**.

4.124 Thallous Sulfate *(38)*—**Official**

Weigh sample contg 0.1–0.15 g Tl_2SO_4 (usually 10 g), transfer to 800 ml Kjeldahl flask, and add 25 ml H_2SO_4 followed by 5–10 ml HNO_3. After first violent reaction ceases, heat until white fumes of H_2SO_4 appear. Add few drops *fuming HNO_3* and continue heating and adding HNO_3 until org. matter is destroyed, as shown by colorless or light yellow soln. Cool, add 10–15 ml H_2O, again cool, and wash contents of flask into 400 ml beaker, continuing washing until vol. is 60–70 ml. Boil several min. to remove all HNO_3, cool, and filter into 400 ml beaker. Wash with hot H_2O until vol. in beaker is 175 ml, neutralize with NH_4OH, and then slightly acidify with H_2SO_4 (1+4). Add 1 g *$NaHSO_3$* to insure reduction of thallic to thallous state. Heat to boiling, add 50 ml 10% KI soln, stir, and let stand overnight. Filter thru tight gooch contg 2 disks S&S 589 white ribbon paper covered by medium pad of asbestos. Wash 4 or 5 times with 10 ml portions 1% KI soln, and finally with absolute alcohol. Dry to constant wt at 105° (1–1.5 hr), and weigh as TlI. From this wt calc. % Tl as Tl_2SO_4, using factor 0.7619.

Warfarin *(39)*—**First Action**

(Applicable to baits contg ca 0.025% and to concentrates contg ca 0.5% warfarin. Not applicable to pelleted baits or baits consisting of cracked corn treated with alc. warfarin soln and aq. sugar soln, and then dried.)

4.125 REAGENTS

(a) *Sodium pyrophosphate soln.*—1%. Dissolve 5 g $Na_4P_2O_7.10H_2O$ in 500 ml H_2O.

(b) *Petroleum ether, purified.*—Ext. 200 ml petr. ether with three 20 ml portions 1% $Na_4P_2O_7$ soln.

(c) *Warfarin std soln.*—10 mmg/ml. Dissolve 100 mg pure warfarin (available from Wisconsin Alumni Research Foundation, 506 N. Walnut St., Madison, Wis.) in 100 ml 1% $Na_4P_2O_7$ soln. Dil. 10 ml to 100 ml with 1% $Na_4P_2O_7$ soln, and dil. 10 ml of second soln to 100 ml with 1% $Na_4P_2O_7$ soln.

4.126 DETERMINATION

Weigh 10 g sample (0.025%) or 0.600 g (0.5%) into 125 ml g-s. flask or 100 ml centrifuge tube and add 50 ml ether from pipet. Stopper tightly and shake on shaking machine ca 30 min. Transfer 5 or 10 ml to centrifuge tube (or centrifuge directly), stopper, and centrifuge 5 min. at high speed or until clear. Take precautions to avoid evapn of ether.

Pipet 10 ml 1% $Na_4P_2O_7$ soln into g-s. 16×150 mm test tube and add from pipet 2 ml of centrifuged ether ext. Stopper and shake vigorously 2 min. Centrifuge at high speed until aq. layer is

clear. Draw off ether layer, including any emulsion that remains, using fine-tip glass tube attached to aspirator. Add ca 2 ml ether, shake vigorously, centrifuge, and completely draw off ether layer. Repeat with second ether extn, and then ext. twice with purified petr. ether in same manner.

Prep. blank soln similarly, using 2 ml ether instead of 2 ml ether ext.

Add sufficient quantity (ca 3 ml) of extd aq. soln to 1 cm silica cell and det. absorbance, A, at 308 mμ against blank soln in Beckman spectrophotometer, model DU, or equiv. Det. absorbance, A' (ca 0.46), of the std warfarin soln against 1% $Na_4P_2O_7$ soln.

$$\% \text{ Warfarin} = (A/A') \times 0.025 \text{ (for baits)}$$
$$\text{or} \times 0.417 \text{ (for concentrates)}.$$

HERBICIDES

Potassium Cyanate (*40*)—Official

4.127 REAGENT

Wash soln.—Satd aq. soln of hydrazodicarbamide. Prep. by mixing some KCNO and semicarbazide.HCl, $NH_2CONHNH_2.HCl$, in H_2O, filter, and wash ppt with H_2O. Transfer ppt to flask, add small quantity H_2O, shake vigorously, and filter. (Solubility of ppt in H_2O is ca 1 part in 6600.)

4.128 DETERMINATION

Transfer sample contg 0.2–0.5 g KCNO to 100 ml beaker, add 20 ml of the wash soln and 1 g semicarbazide.HCl, and let stand 24 hr. Filter hydrazodicarbamide ($NH_2CONHNHCONH_2$) on gooch or fine fritted glass crucible, wash with 10 ml of the wash soln, and dry at 100° to constant wt. Wt residue×0.6868 = KCNO.

4.129 2,4-Dichlorophenoxyacetic Acid (2,4-D) (*41*)—Official

(a) *In preparations of free acid with no insoluble carrier.*—Weigh 1 g sample into 250 ml erlenmeyer, dissolve in 75 ml neutral alcohol, and titr. with 0.1*N* NaOH, using 1 ml phthln. (1 ml 1% alc. thymolphthalein soln may be substituted, provided this indicator has been used to stdze the alkali.) 1 ml 0.1*N* NaOH = 0.0221 g 2,4-dichlorophenoxyacetic acid.

(b) *In herbicides containing free acid and insoluble carrier.*—Weigh sample equiv. to 1 g of the acid into 250 ml beaker, add 25 ml 1*N* NaOH and 50 ml H_2O, warm and stir 15 min. to dissolve acid, and adjust to room temp. Filter thru paper into 250 ml separator and wash any insol. matter, collecting washings in separator. Neutralize contents of separator with 10% H_2SO_4, add 10 ml excess, and ext. with two 75 ml portions ether. Combine 2 ether exts in separator, wash free from H_2SO_4 with three 10 ml portions H_2O, and filter thru cotton plug (previously satd with ether) into 400 ml beaker. Rinse separator with ether, and filter rinsings thru the cotton into beaker. To contents of beaker add 25 ml H_2O and few boiling chips, evap. on steam bath until ca 25 ml ether remains, and then remove balance of ether at room temp. in air current. To residual aq. soln add 100 ml neutral alcohol and titr. with 0.1*N* NaOH as in (a).

(c) *In herbicides containing salts of 2,4-D.*—Weigh sample equiv. to ca 1 g of the free acid and dissolve in 50 ml H_2O. If insol. carrier is present, filter thru paper and wash residue. Transfer clear soln to 250 ml separator, and proceed as in (b), beginning "Neutralize contents of separator . . . "

Total Chlorine in Compounds of 2,4-D and 2,4,5-Trichlorophenoxyacetic Acid (2,4,5-T) in Liquid Herbicides

Parr Bomb-Boric Anhydride Method (42)—Official

(CAUTION: Observe precautions necessary in Parr bomb ignitions.)

4.130 REAGENTS

Boric anhydride.—Eastman or Fisher material has been found satisfactory; or prep. by heating H_3BO_3 at 120–220° ca 2 weeks.

4.131 PREPARATION OF SAMPLE

(a) *Esters (Official).*—To 2.5 g B_2O_3 in 42 ml Parr bomb, elec. ignition type, add, from small weighing buret, ca 0.2–0.6 g sample (0.030–0.034 g Cl is convenient). Never take sample >0.6 g. Samples from 0.4 to 0.6 g will burn intensely without accelerator. When sample is <0.4 g add 99% isopropyl alcohol as accelerator so that total org. matter approaches 0.5 g, but do not use >0.25 g isopropyl alcohol. (Intense burn of sample is required for total Cl recovery.)

(b) *Amine salts (First Action).*—Weigh ca 0.5 g sample from weighing bottle or buret into cup of 42 ml Parr elec. ignition bomb. Add 5–10 drops NaOH soln (1+1), avoiding excess which makes residue difficult to break up, to decompose amine radical and make Na salt. Place in oven 30–60 min. at 100°. Cool cup, add 0.2 ml 99% isopropyl alcohol, break up softened residue, and add 2.5 g B_2O_3.

4.132 DETERMINATION

Mix mixt. in cup well with thin stirring rod. Measure 15 g *calorimetric grade* Na_2O_2 with std measuring dipper, add small portion to contents of bomb, and stir. Add balance of Na_2O_2, and thoroly mix by stirring with rod. Withdraw rod and brush free of adhering particles. Quickly cut or break off lower 1.5" of rod and imbed in fusion

mixt. Prep. head by heating fuse wire momentarily in flame and immersing it in small quantity *sucrose*. (1 mg sucrose is enough to start combustion.) Assemble bomb and ignite in usual manner.

Place ca 100 ml H_2O in 600 ml beaker and heat nearly to boiling. After cooling bomb, dismantle and dip cover into the hot H_2O to dissolve any fusion mixt. on underside. Wash cover with fine jet of H_2O, catching washings in beaker. With tongs, lay fusion cup on side in same beaker of hot H_2O, covering it immediately with watch glass. After fused material dissolves, remove cup and rinse with hot H_2O, cool soln, add several drops phthln, neutralize with HNO_3, and add 5 ml excess. Det. Cl by Volhard method, **4.161**(a), or by electrometric titrn, **4.161**(c).

Det. blank which includes all reagents used. Cl $\times$3.117 = 2,4-D acid; $\times$2.402 = 2,4,5-T acid.

Volatility of Ester Forms of Hormone Type Herbicides—First Action

4.133 MATERIAL

(a) *Paper bags*.—No. 20 to open with flat bottom. Close with paper clips.

(b) *Filter paper*.—7 cm diam.

(c) *Bacteriological loop*.—0.01 ml. Wash with acetone after each application or heat to cherry red in flame.

(d) *Test plants*.—Actively-growing tomato seedlings 2.5–3″ high in 3–4″ pots.

(e) *Formulation to be tested*.—Use 0.01 ml aliquot of 4 lb/gallon formulation or equiv. vol. of other concns.

(f) *High and low volatile ester stds*.—Use butyl ester of 2,4-D as high volatile ester and tetrahydrofurfural ester of 2,4-D as low volatile ester with same wt of acid/gallon as formulations to be tested.

4.134 OPERATING TECHNIC

Open bags with flat bottom and place plant toward one side on bottom of bag. Apply 0.01 ml of formulation to middle of filter paper by means of bacteriological loop, and for controls, apply 0.01 ml solvent only. Place treated paper in bottom of bag. Do not touch treated part of paper against plant, sides of bag, or pot. Close bag by folding top, secure with clips, and let stand 24 hr at 85–110°F.

Use 3 plants per treatment and 3 for controls. Repeat test on another day.

Remove plants from bag, let stand 24 hours, and read curvature (stem bending, epinasty) response. (Fold and discard used bags to prevent contamination.) Rate plants according to scale 1 to 6. (*See* Fig. 7.) To detect small differences between low volatile esters, or differences between 2,4-D and 2,4,5-T types, hold plants 7 days after treatment to allow time for modified leaves or stem lesions to develop. Absence of such responses indicates that formulation was a low volatile 2,4,5-T ester.

ORGANIC MERCURIAL SEED DISINFECTANTS

Mercury

Volatilization Method (43)—Official

4.135 APPARATUS

App., Fig. 8, consists of 2 flanged crucibles that can be clamped mouth to mouth by means of 2 rings and screws. Lower crucible is made of Fe, ca 20 ml capacity, 32 mm high, 40 mm top opening machined to make flange; upper Au flanged crucible, 32 mm bottom diam., 40 mm top opening with 2.5 mm flange (side walls 0.25–0.4 mm, flange 1.1–1.3 mm thick) is available from

FIG. 7.—RESPONSE SCALE OF TEST PLANTS TO ESTER FORMS OF HORMONE TYPE HERBICIDES

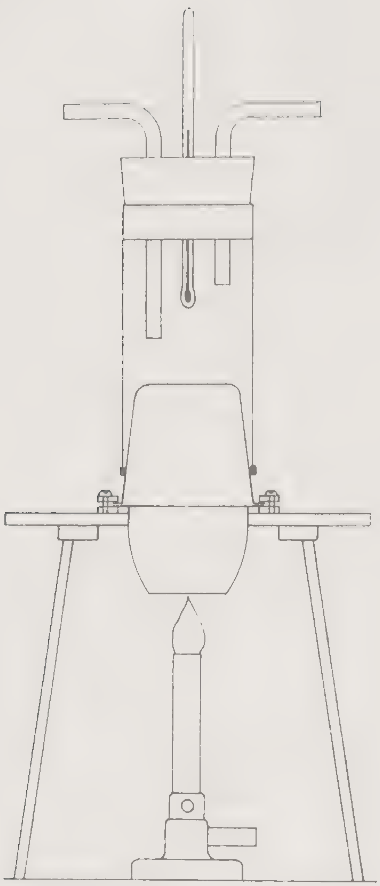

FIG. 8.—APPARATUS USED IN VOLATILIZATION METHOD FOR DETERMINATION OF MERCURY

American Platinum Works, Newark 5, N. J. Opening of Au crucible is slightly larger than that of the other, so that the Hg will not tend to lodge in joint between the 2 flanges. Au crucible is fitted with glass cooling device 42 mm i.d.×110 mm long thru which H_2O may be slowly circulated. It is attached to crucible by Gooch tubing. Assembled app. rests on asbestos board having hole just large enough to receive crucible.

4.136 DETERMINATION

Weigh 1 g sample into the Fe crucible and mix thoroly with 5 g anhyd. Na_2CO_3. Cover mixt. with thin layer Na_2CO_3 and then with 10 g *finely powd. $BaCO_3$*. Put weighed Au crucible in place, clamp two crucibles together, set Fe crucible in place in asbestos board, start cooling H_2O, and gently heat Fe crucible. Do not run H_2O too fast because Hg amalgamates best with Au crucible if temp. is allowed to rise to ca 50°. Heat below redness 30 min., cool, remove Au crucible, wash with alcohol, dry with heat of hand, and place in $CaCl_2$ desiccator to constant wt. Calc. increase in wt of Au crucible as % metallic Hg in sample. If product contains >12% Hg, use <1 g because Au crucible can safely retain only ca 0.12 g Hg. Remove Hg from Au crucible, prior to another detn, by short ignition at dull red heat under hood having good draft. (Crucible melts in full heat of Bunsen burner.)

Precipitation Method (44)—Official

4.137 DETERMINATION

Place 0.5–2.0 g sample, depending on quantity of Hg present, in 200 ml erlenmeyer connected thru $\mathfrak{F}$ joint to air condenser. Add 10 ml H_2SO_4, connect flask to condenser, and rotate so acid will wet entire sample. Add 3–5 ml *30% H_2O_2* dropwise thru condenser tube, and rotate flask to mix. After active reaction subsides, heat over low flame 15–20 min., add 5 ml more of the H_2O_2, and continue heating until all org. matter is destroyed (indicated by clear soln), adding more H_2O_2 if necessary. Remove flask from heat, let cool, wash down condenser, and transfer contents to beaker, filtering if necessary. Dil. to ca 200 ml and destroy excess H_2O_2 by titrn with 3% $KMnO_4$ soln. Ppt the Hg with *H_2S*, filter thru weighed gooch, and dry at 105–110°. Ext. dried ppt with CS_2 to remove any pptd S, again dry, and weigh. HgS ×0.8622 = Hg.

SODIUM HYPOCHLORITE SOLUTIONS (45)

Sodium Hypochlorite

Arsenious Oxide Titration Method—Official

4.138 REAGENTS

(a) *Arsenious oxide soln.*—0.1*N*. Dissolve exactly 2.473 g pure As_2O_3 in beaker by boiling with 150–200 ml H_2O contg 10 ml H_2SO_4. Cool, add phthln, neutralize with NaOH soln, adjust to faint acid reaction, transfer to 500 ml vol. flask, and dil. to mark.

(b) *Iodine std soln.*—Prep. as in **42.016.** Stdze against (a).

4.139 DETERMINATION

Transfer 20 ml sample to 1 L vol. flask and dil. to vol. Pipet 50 ml aliquot of mixt. into 200 ml erlenmeyer. Add excess As_2O_3 soln and then decided excess $NaHCO_3$. Titr. excess As_2O_3 with the I soln, using starch soln, **4.004(f)**, or the I as its own indicator. Subtract vol. I soln, corrected to 0.1*N*, from vol. As_2O_3 soln used, and from this value and sp. gr. of soln, calc. % NaOCl. 1 ml 0.1*N* As_2O_3 = 0.003723 g NaOCl.

4.140 **Available Chlorine—Official**

Calc. % available Cl from titrn, **4.139.** 1 ml 0.1*N* As_2O_3 = 0.003546 g available Cl.

4.141 **Chloride Chlorine—Official**

Pipet 50 ml aliquot prepd soln, **4.139,** into 200 ml erlenmeyer and add slight excess As_2O_3 soln, **4.138(a)**, calcd from NaOCl titrn; add slight excess HNO_3, neutralize with *$CaCO_3$*, and titr.

with $0.1N$ $AgNO_3$, **4.083**, using K_2CrO_4 soln, **2.082(b)**, or the Ag_3AsO_4 formed in soln, as indicator. Det. blank on reagents and correct for any Cl found. From this corrected titrn and sp. gr. of sample, calc. % Cl. From this value subtract ½ the % available Cl. Difference = % chloride Cl.

4.142 Sodium Hydroxide—First Action

Stdze pH meter equipped with calomel and glass electrodes, using std pH 6.9 buffer soln, **42.007(d)**.

Place 50 ml 10% $BaCl_2.2H_2O$ soln and 30 ml 3% H_2O_2 soln in 250 ml beaker. Neutralize to pH 7.5 with ca $0.1N$ NaOH, using pH meter. Add to this soln from pipet 10.0 ml sample, stir vigorously 1 min., and titr. to pH 7.5 with stdzd $0.1N$ HCl, using pH meter.

$$\% \text{ NaOH} = \frac{\text{ml HCl} \times \text{normality} \times 4.0}{\text{ml sample} \times \text{sp. gr.}}$$

Carbon Dioxide—Official

4.143 APPARATUS

Connect evolution flask, to which is attached dropping funnel protected by tube contg soda lime, to condenser or Kjeldahl distg trap, connected in turn to 2 wash bottles contg 10% KI soln. Use glass beads or other device in wash bottles to cause gas to flow slowly thru liquid. End train with Meyer absorption tube contg $0.1N$ $Ba(OH)_2$.

4.144 DETERMINATION

Pipet suitable portion of sample (5–20 ml, depending upon quantity of CO_2 present) into evolution flask, and attach flask to train. Place 50 ml $0.1N$ $Ba(OH)_2$ in Meyer tube, and add 35–50 ml 3% *H_2O_2 soln* (or enough to reduce hypochlorite) thru dropping funnel into evolution flask. After action due to H_2O_2 ceases, add 30 ml HCl (1+3), heat flask to boiling, and draw air slowly thru app. (Evolved gases are freed from Cl by KI in wash bottles, and the CO_2 is absorbed in the std $Ba(OH)_2$ in Meyer tube.) Draw air thru app. 20 min., disconnect Meyer tube, and pour contents into beaker. Wash out tube, adding washings to contents of beaker. Filter, wash, and titr. filtrate and washings with $0.1N$ HCl, using phthln. From ml $0.1N$ $Ba(OH)_2$ used and sp. gr. of sample, calc. % CO_2. 1 ml $0.1N$ $Ba(OH)_2$ = 0.00220 g CO_2.

CALCIUM HYPOCHLORITE AND BLEACHING POWDER (*45*)

Available Chlorine

4.145 *Arsenious Oxide Titration Method—Official*

Weigh 5–10 g thoroly mixed sample into porcelain mortar, add 30–40 ml H_2O, and triturate to smooth cream (high-test $Ca(OCl)_2$ will dissolve readily and not form a cream). Add more H_2O, stir well with pestle, and let insol. residue settle few moments. Pour mixt. off into 1 L vol. flask, add more H_2O, and thoroly triturate sample and pour off as before. Repeat operation until all material is transferred to flask. Rinse mortar and pestle, catch wash H_2O in flask, dil. to mark, and mix. Without letting material settle, pipet 25–50 ml aliquot into 200 ml erlenmeyer. Add excess std As_2O_3 soln, **4.138(a)**, and then decided excess of $NaHCO_3$. Titr. excess As_2O_3 with std I soln, **4.138(b)**, using starch soln, **4.004(f)**, or the I as its own indicator. Subtract vol. I soln, corrected to $0.1N$, from vol. As_2O_3 soln used, and calc. % available Cl. 1 ml $0.1N$ As_2O_3 = 0.003546 g available Cl.

CHLORAMINE T (*45*)

Active Chlorine

Arsenious Oxide Titration Method—Official

4.146 REAGENTS—*See* **4.138**

4.147 DETERMINATION

Transfer 0.5 g sample to 300–500 ml erlenmeyer, dissolve in 50 ml H_2O, and add excess of std As_2O_3 soln, **4.138(a)**, and 5 ml H_2SO_4 (1+4). Add decided excess $NaHCO_3$ and titr. excess As_2O_3 with std I soln, **4.138(b)**, using starch soln, **4.004(f)**, or I as its own indicator. From this titrn calc. active Cl in sample. 1 ml $0.1N$ As_2O_3 soln = 0.001773 g active Cl. (To convert active Cl to available Cl, multiply active Cl by 2.)

4.148 Total Chlorine—Official

Dissolve 0.5 g sample in 50 ml H_2O in erlenmeyer and add slight excess of the std As_2O_3 soln as calcd from active Cl titrn, **4.147.** Add 5 ml HNO_3 (1+4), neutralize with *$CaCO_3$*, and titr. with std $AgNO_3$, **4.083**, using K_2CrO_4, **2.082(b)**, as indicator. Det. blank on reagents and correct for any Cl found. From corrected titrn calc. % total Cl in sample. 1 ml $0.1N$ $AgNO_3$ = 0.003546 g Cl. If total Cl exceeds active Cl, NaCl is indicated.

4.149 Sodium—Official

Weigh 0.5 g sample in SiO_2 or porcelain dish and add ca 25 ml H_2O and 3–5 ml H_2SO_4 (1+4). Evap. to sirupy consistency on steam bath and finally to dryness on hot plate. Ignite at full heat of Bunsen burner, cool, and weigh as Na_2SO_4. (Residue should be completely sol. in H_2O and should show no turbidity with NH_4OH and $(NH_4)_2CO_3$.) Test for Na in flame. If residue meets these tests it may be considered pure Na_2SO_4. From wt residue calc. % Na in sample.

QUATERNARY AMMONIUM COMPOUNDS

Chloride—First Action

4.150 *Potentiometric Titration Method*

Transfer sample contg 30–35 mg Cl to 600 ml beaker, dil. to 200 ml with H_2O, and add 5 ml HNO_3 (1+1). Add just enough acetone to dissolve ppt that forms and titr. with 0.1N $AgNO_3$, using potentiometric titrimeter (Fisher Titrimeter or equiv.). Calc. % Cl (1 ml 0.1N $AgNO_3$ = 3.546 mg Cl) and equiv. % quaternary NH_4 salt.

Adsorption Indicator Method

4.151 REAGENTS

(a) *Bromothymol blue indicator.*—Dissolve 1 g indicator in 500 ml 50% alcohol.

(b) *Dichlorofluorescein soln.*—0.1%. Dissolve 100 mg indicator in 100 ml 70% alcohol.

4.152 DETERMINATION

Transfer sample contg 30–140 mg Cl (usually ca 1 g quaternary NH_4 salt) into 300 ml erlenmeyer, dil. to 75 ml with H_2O, and add 25 ml isopropyl alcohol. Neutralize if necessary with HOAc (1+9), using 1 drop bromothymol blue (pH 4–6). Add 10 drops dichlorofluorescein, and titr. with 0.1N $AgNO_3$, avoiding direct sunlight. Ppt becomes red at end point and may flocculate just before end point. Calc. % Cl and equiv. % quaternary NH_4 salt.

TECHNICAL ALLETHRIN (*46*)—FIRST ACTION

4.153 PRINCIPLES

Allethrin reacts quantitatively with ethylenediamine to form chrysanthemum monocarboxylic acid which is detd by titrn with std NaOMe in pyridine. Chrysanthemum monocarboxylic acid, anhydride, and acid chloride interfere quantitatively and are detd independently.

4.154 REAGENTS

(a) *Absolute alcohol.*—Special Denatured Formula No. 2-B is satisfactory.

(b) *Methanolic hydrochloric acid std soln.*—0.1N. Dil. 17 ml HCl (1+1) to 1 L with anhyd. MeOH. Stdze against std 0.1N NaOH, using phthln. If used at temp., T, different from that at which stdzed, T_0, calc. corrected normality $= N[1-0.001(T-T_0)]$.

(c) *Sodium methylate std soln.*—0.1N in pyridine. Transfer 50 ml 2N NaOMe to 1 L bottle contg 75 ml anhyd. MeOH and dil. to 1 L with redistd pyridine. Stdze against NBS benzoic acid, using pyridine as solvent and thymolphthalein, (i), as indicator. Dispense from 50 ml automatic buret with vents connected to Ascarite tubes. Stdze daily against std methanolic HCl, (b).

(d) *Methanolic potassium hydroxide std soln.*—0.02N.

(e) *Morpholine soln.*—Transfer 8.7 ml redistd morpholine to 1 L bottle and dil. to 1 L with anhyd. MeOH. Fit bottle with 2-hole rubber stopper; thru 1 hole insert 20 ml pipet so that tip extends below surface of liquid, and thru other hole insert short piece of glass tubing to which is attached aspirator bulb.

(f) *Ethylenediamine.*—Redistd commercial grade contg <3% H_2O. Dispense from automatic buret with vents connected to Ascarite tubes.

(g) *Dimethyl yellow-methylene blue mixed indicator.*—Dissolve 1 g dimethyl yellow (*p*-dimethylaminoazobenzene) and 0.1 g methylene blue in 125 ml anhyd. MeOH.

(h) *α-Naphtholbenzein indicator.*—1% alc. soln.

(i) *Thymolphthalein indicator.*—1% pyridine soln.

4.155 DETERMINATION OF CHRYSANTHEMUM MONOCARBOXYLIC ACID CHLORIDE

Add 8–10 drops of the mixed indicator, (g), to ca 150 ml anhyd. MeOH and add 0.1N HCl, (b), dropwise until soln appears reddish-brown by transmitted light. Add 0.02N KOH, (d), dropwise until appearance of first green color. Transfer 25 ml of this soln to each of three 125 ml g-s. erlenmeyers, reserving 1 flask as reference color for end point. Into each of other flasks add 1.5–2.5 g sample from weighing pipet, swirling flask while adding sample. Within 5 min., titr. with 0.02N KOH, (d), to first green end point, using blank as reference color. Calc. milliequiv. chrysanthemum monocarboxylic acid chloride/g sample, $C = A \times N$/g sample, where A = ml N normal KOH required; $C \times 18.67$ = % chrysanthemum monocarboxylic acid chloride.

4.156 DETERMINATION OF CHRYSANTHEMUM MONOCARBOXYLIC ACID

Transfer 25 ml anhyd. EtOH to each of two 125 ml g-s. erlenmeyers, add 8–9 drops α-naphtholbenzein indicator, and cool to 0° in ice bath. Neutralize by adding 0.02N NaOH dropwise to bright green end point. To each flask add 1.5–2.5 g sample from weighing pipet. Immediately titr. with 0.02N NaOH, **42.032**, to first bright green end point. Calc. milliequiv. chrysanthemum monocarboxylic acid and acid chloride/g sample: $D = A \times N$/g sample, where A = ml N normal NaOH required; $(D-C) \times 16.82$ = % chrysanthemum monocarboxylic acid.

4.157 DETERMINATION OF CHRYSANTHEMUM MONOCARBOXYLIC ANHYDRIDE

Pipet 20 ml morpholine soln, (e), into each of four 250 ml erlenmeyers, using same pipet. Fill

pipet by exerting pressure in bottle with aspirator bulb. Reserve 2 flasks for blanks; into each of other flasks add 1.5–2.5 g sample from weighing pipet. Swirl flasks and let samples and blanks stand at room temp. 5 min. Add 4–5 drops mixed indicator (**g**), to each flask and titr. with $0.1N$ HCl, (**b**), until color changes from green to faint red when viewed by transmitted light. Calc. milliequiv. chrysanthemum monocarboxylic anhydride/g sample: $E=(B-A)\times N/\text{g}$ sample, where A = ml N normal HCl required for sample, and B = ml N normal HCl required for blank; $(E-2C)\times 31.84=\%$ chrysanthemum monocarboxylic anhydride.

4.158 DETERMINATION OF ALLETHRIN

Add sample contg 0.8–1.1 g allethrin to each of two 250 ml erlenmeyers from weighing pipet. To each of 2 flasks as blanks and to samples add 25 ml ethylenediamine, (**f**), with swirling. Let samples and blanks stand 2 hr at $25\pm 2°$. Wash down sides of flasks with 50 ml redistd pyridine. To each flask add 6–10 drops thymolphthalein indicator, (**i**), and titr. with $0.1N$ NaOMe, (**c**), to first permanent blue-green end point. (With colorless samples, first blue end point may be used.) Calc. milliequiv. allethrin/g sample: $F=(A-B)\times N/\text{g}$ sample, where A = ml N normal NaOMe required for sample, and B = av. ml N normal NaOMe required for blank; $(F+C-D-E)\times 30.24=\%$ allethrin.

2,2-BIS(*p*-CHLOROPHENYL)-1,1,1-TRICHLOROETHANE (DDT)

Total Benzene-Soluble Chlorine Method (47)—Official

(Applicable in absence of other org. Cl compounds)

4.159 REAGENTS

(**a**) *Benzene.*—Thiophene- and Cl-free.

(**b**) *Metallic sodium.*—Ribbons or small pieces.

(**c**) *Decolorizing carbon.*—Test for presence of Cl by heating with HNO_3 (1+4), filtering, and adding $AgNO_3$ soln to filtrate. If Cl is present, wash with the HNO_3 until washings are Cl-free.

NOTE: Run blank on all reagents, limiting the $0.1N$ $AgNO_3$ to 5 ml. Use the H_2O_2 and isoamyl alcohol-ether extn method on dispersible powders or sprays that contain surface active agents or other ingredients that react with $AgNO_3$.

4.160 PREPARATION OF SOLUTION

(**a**) *In technical grade DDT.*—Weigh sample contg ca 1 g DDT and transfer to 250 ml vol. flask. Dissolve sample in 10 ml of the benzene; then dil. to vol. with *99% isopropanol*. Transfer 25 ml aliquot to 250–500 ml ₸ erlenmeyer. (Direct weighing of sample may be substituted, provided it does not introduce error $>0.1\%$.)

Add 2.5 g of the Na and shake flask to mix sample with the isopropanol. Connect flask to reflux condenser and boil gently at least 30 min., shaking flask occasionally. Eliminate excess Na by cautiously adding 10 ml 50% isopropanol thru condenser at rate of 1–2 drops/sec. Disconnect condenser, add 60 ml H_2O, boil soln ca 30 min. to expel isopropanol, and proceed as in **4.161**(**a**), (**b**), (**c**), or (**d**).

(**b**) *In dusting mixtures containing DDT in absence of organic matter.*—Weigh sample contg ca 0.75 g DDT, transfer to 100–200 ml vol. flask, and add exactly 100 ml of the benzene. Shake until DDT dissolves and soln is well mixed. Let settle and transfer 10 ml aliquot to 250–500 ml ₸ erlenmeyer.

Evap. on steam bath to remove most of benzene. (It is not desirable to evap. to dryness, as DDT may decompose with loss of HCl.) Add 25 ml *99% isopropanol* and proceed as in (**a**), second par.

If free S is present, proceed as in (**f**), beginning "Add 5 ml *30%* H_2O_2 . . . "

(**c**) *In dusting mixtures in presence of organic matter (coloring matter, plant resins, etc.).*—Weigh sample contg ca 0.75 g DDT, transfer to 100–200 ml vol. flask, and add 0.5–1.0 g of the decolorizing C and exactly 100 ml of the benzene. Filter into narrow-neck flask thru fast qual. paper without suction, keeping funnel covered with watch glass to avoid evapn loss. Transfer 10 ml aliquot to 250–500 ml ₸ erlenmeyer. Proceed as in (**b**), second par. Before detg Cl remove org. matter as follows:

Cool, add 2 or 3 drops phthln, and neutralize by adding HNO_3 (1+1) dropwise, then 10 ml excess. Cool, if necessary, to room temp., transfer contents of flask and aq. washings to small separator, and shake with 15 ml *isoamyl alcohol*-ether (1+1). Drain aq. layer into second separator and ext. again with 15 ml of the isoamyl alcohol-ether mixt. Drain aq. layer into 250 ml beaker. Wash the 2 exts successively with 10 ml H_2O, and repeat second washing with another 10 ml H_2O. Combine the aq. wash solns with aq. soln in beaker. Det. Cl by one of following methods:

(1) Proceed as in **4.161**(**a**), beginning "Add slight excess $0.1N$ $AgNO_3$ . . . "

(2) Proceed as in **4.161**(**b**), beginning "Add $0.1N$ $AgNO_3$ . . . "

(3) Proceed as in **4.161**(**c**), beginning "Cool flask to room temp . . . "

(4) Add 2 or 3 drops phthln to sample, make alk. by adding $1N$ NaOH, and proceed as in **4.161**(**d**), beginning "transfer contents to Pt dish."

(**d**) *In mineral oil sprays in absence of organic matter (plant extractive material, organic thiocyanates).*—Transfer weighed sample contg 0.065

0.075 g DDT to 250–500 ml ₮ flask. Add 25 ml *99% isopropanol* and proceed as in (**a**), second par.

NOTE: If DDT content is <2%, use isoamyl alcohol-ether extn, (**c**), second par., to remove excess oil.

Proceed as in **4.161**(**a**), (**b**), (**c**), or (**d**).

(**e**) *In mineral oil sprays in presence of organic matter (plant extractive material from pyrethrum or derris and/or cubé.)*—Proceed as in NOTE under (**d**).

(**f**) *In mineral oil sprays in presence of organic thiocyanates with or without plant extractive material.*—Transfer sample contg 0.065–0.075 g DDT to 250–500 ml ₮ erlenmeyer. Add 25 ml *99% isopropanol* and proceed as in (**a**), second par. thru "add 60 ml H_2O . . ." Then add 5 ml *30% H_2O_2*, few drops at time, thru top of condenser, heat mixt. in flask to boiling, and boil 15 min. Add 5 ml more of the H_2O_2 and again boil 15 min. Add 15 ml more of the H_2O_2, disconnect reflux condenser, and boil 15–30 min. to expel isopropanol. Proceed as in (**c**), second par.

(**g**) *In emulsions (solvent, emulsifier, and water).*—Weigh well-mixed sample contg ca 0.75 g DDT in weighing bottle. Wash into 100 ml vol. flask and dil. to vol. with isopropanol. Transfer 10 ml aliquot to 250–500 ml ₮ erlenmeyer. Expel isopropanol and H_2O on steam bath in air current. If drops of H_2O still remain, add 10 ml isopropanol and repeat evapn. Add 25 ml *99% isopropanol* and proceed as in (**a**), second par.

NOTE: If S is brought into the soln as by decomposition of the emulsifier, proceed as in (**f**), beginning "add 5 ml *30% H_2O_2* . . ."

4.161 DETERMINATION

(**a**) Cool flask and transfer contents to 250 ml beaker. Add 2–3 drops phthln and neutralize with HNO_3 (1+1); then add 10 ml excess. Add slight excess 0.1*N* $AgNO_3$, **42.029**, and coagulate pptd AgCl by digesting on steam bath 30 min., stirring frequently. Cool, filter thru fast qual. paper, and wash thoroly with H_2O. Add 5 ml satd Fe alum soln, **4.015**(**e**), and det. excess $AgNO_3$ in filtrate by titrn with 0.1*N* KCNS, **42.028**(**b**). Subtract quantity $AgNO_3$ found in filtrate from that originally added. Difference is that required to combine with Cl in the DDT. 1 ml 0.1*N* $AgNO_3$ = 0.003546 g Cl. Cl×2 = DDT.

(**b**) Cool flask, add 2–3 drops phthln soln, and neutralize with HNO_3 (1+1); then add 10 ml excess. Add 0.1*N* $AgNO_3$ from buret in excess of quantity necessary to ppt all Cl; then add 5 ml *nitrobenzene* and 0.5 g *$Fe_2(SO_4)_3$* and swirl flask to coagulate ppt. Back-titr. excess $AgNO_3$ with 0.1*N* KCNS to faint pink. Cross-titr. with both std solns, crossing end point in each direction to assure results. Calc. % DDT as in (**a**) from quantity of $AgNO_3$.

(**c**) Cool flask, add 2–3 drops phthln soln, neutralize with HNO_3 (1+1), and add 6 ml excess. Cool flask to room temp. and transfer contents to 400 ml beaker. (Vol. should be 200–250 ml). Titr. Cl with 0.1*N* $AgNO_3$ potentiometrically, using Ag-AgCl electrodes (Fisher Titrimeter or equiv.). Calc. % DDT as in (**a**).

NOTE: When this method is used, decolorizing C step in **4.160**(**c**), and isoamyl alcohol-ether extn in **4.160**(**c**), (**d**), and (**e**), may be omitted.

(**d**) Cool flask and transfer contents to Pt dish. Evap. to dryness and ignite as thoroly as possible at temp. not exceeding dull red. Ext. with hot H_2O, filter, and wash. Return residue to Pt dish and ignite to ash; dissolve in HNO_3 (1+4), filter from any insol. residue, wash thoroly, and add this soln to aq. ext. Add 0.1*N* $AgNO_3$, avoiding more than slight excess. Heat to boiling, protect from light, and let stand until ppt coagulates. Filter on weighed gooch, previously heated to 140–150°, and wash with hot H_2O, testing filtrate to prove excess of $AgNO_3$. Dry AgCl at 140–150°, cool, and weigh. Calc. % Cl and from this calc. % DDT as in (**a**).

NOTE: Run blank on all reagents, limiting 0.1*N* $AgNO_3$ to 5 ml.

Infrared Method (48)—First Action

4.162 REAGENT

DDT std soln.—Weigh 0.250 g technical DDT (Insecticide Reference Standard DDT available from Nutritional Biochemical Corp., 21010 Miles Ave., Cleveland 28, Ohio, is satisfactory) into 50 ml vol. flask or g-s. container and add exactly 25 ml CS_2. If sample to be analyzed contains S, add wt of S expected in portion of sample to be taken for analysis. Shake to dissolve and add small quantity of anhyd. Na_2SO_4. Centrifuge portion of soln if it is not clear.

4.163 DETERMINATION

Weigh sample contg ca 0.25 g DDT into 50 ml vol. flask and add exactly 25 ml CS_2 and small quantity of anhyd. Na_2SO_4. Let stand at least 30 min. with occasional shaking. Transfer portion to g-s. test tube and centrifuge short time. Transfer to NaCl cell and scan with Perkin-Elmer model 21 spectrophotometer, or equiv., using following settings and conditions: cell, 0.5 mm; no compensation; balance instrument without cells in place; region, 8.5–10.5 μ; resolution, 960 (Program); speed, 4; filter, out; gain, adjusted.

Scan std soln in same manner.

Measure absorbance of DDT peak at 9.83 μ

with baseline from 9.4 μ to 10.2 μ, and calc. % DDT.

HEXACHLOROCYCLOHEXANE ("BENZENE HEXACHLORIDE") (BHC)

Gamma Isomer (Lindane)

Partition Chromatographic Method (49)—Official

4.164 APPARATUS

(**a**) *Partition column.*—Column and O type reduction valve are shown in Fig. 9. Construct column of heavy-wall Pyrex tubing ca 3.5 mm thick, 90 cm long×2.5 cm diam. Seal coarse porosity fritted glass disk in place and attach No. 18/9 ball and socket joint 5 cm below disk. Supply pressure from laboratory supply line. (Column available from Scientific Glass App. Co., Bloomfield, N. J., specifying catalog No. J-1660-4 constructed from heavy rather than std wall tubing.)

(**b**) *Solvent evaporator.*—Fig. 9. Fractions are evapd to dryness under reduced pressure at 60°, with aid of H_2O pump. Solvent is recovered in trap consisting of Kjeldahl flask immersed in mixt. of NaCl and ice.

(**c**) *Melting point apparatus.*—Use Thiele m.p. app. equipped with mechanical stirrer. App. shown in Fig. 72, **35.127**, or Hershberg modification (*50*) (available from Ace Glass, Inc., Vineland, N. J., catalog No. 76) is suitable.

(**d**) *Thermometer.*—Precision grade, meeting NBS specifications; partial immersion; range 90–120° in 0.2° subdivisions. Calibrated by, or calibrated against thermometer checked by, NBS.

(**e**) *Melting point tubes.*—1–2 mm capillary tubes of uniform wall thickness and diam.

4.165 REAGENTS

(**a**) *n-Hexane.*—Commercial grade, distd before use.

(**b**) *Nitromethane.*—Reflux commercial grade material 4 hr and distill. No visible residue is left after evapn of 10 ml purified material.

(**c**) *Silicic acid.*—Use Mallinckrodt reagent grade (for chromatography) which meets following requirements: When column prepd as in **4.167** is used for detn on sample contg known amount of γ-isomer, flow rate and packing characteristics should be similar to those of an H_2SiO_3 known to be satisfactory, and recovery of γ-BHC should be within ±3% of the γ-BHC content.

(**d**) *Dye soln.*—Dissolve 25 mg D&C Violet No. 2 (1-hydroxy-4-*p*-toluino-anthraquinone) in 50 ml mobile solvent and store in g-s. bottle. (Available from Pylam Products, 799 Greenwich St., New York 14, N. Y.)

(**e**) *Mobile solvent.*—Satd soln nitromethane in *n*-hexane. Vigorously shake 2 L *n*-hexane with excess nitromethane in g-s. bottle. Decant mobile solvent from nitromethane as needed.

4.166 PREPARATION OF SAMPLE

(**a**) *Powders with >10% γ-BHC.*—Crush and thoroly mix sample with mortar and pestle. Weigh enough sample into tared 125 ml erlenmeyer to provide ca 0.2 g γ-isomer after extg and aliquoting. Add 25 ml mobile solvent, heat just to boiling on steam bath, and cool to room temp., shaking occasionally. Decant and ext. thru büchner with ca 34 mm fritted disk, medium porosity, into 100 ml Kohlrausch flask, with gentle suction. Re-ext. residue in flask, using 10 ml mobile solvent. Wash residue and flask with five 10 ml

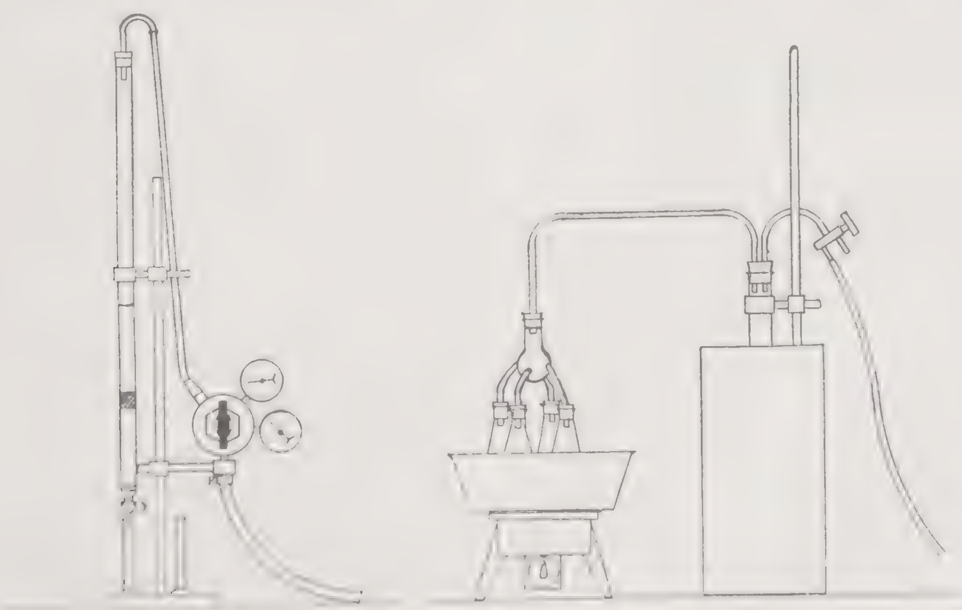

FIG. 9.—PARTITION COLUMN AND SOLVENT EVAPORATOR

portions of cold mobile solvent, decanting each wash thru büchner. (Extn may also be performed in filter-beaker such as catalog No. JH-278 of Scientific Glass App. Co., filtering into 100 ml vol. flask.) To contents of flask add 2 ml dye soln and dil. to mark with mobile solvent.

(b) *Dusts containing <10% γ-BHC.*—Weigh enough sample to provide 1.75–2.00 g γ-isomer. Transfer to Soxhlet extractor and ext. overnight with ether. Evap. most of ether on steam bath and evap. remainder at room temp. under vac. Ext. γ-isomer from residue with mobile solvent as in (a).

4.167 PREPARATION OF COLUMN

Transfer 100 ± 0.5 g H_2SiO_3 to high-speed blender, add 300 ml mobile solvent, and with mixing, add 55 ml nitromethane. After mixing 15–30 sec. pour mixt. into column thru glass funnel. Stir slurry with long glass stirring rod to displace air bubbles. Wash down sides of column with few ml mobile solvent and apply 5 lb pressure to pack column and force out excess solvent; tap column gently to aid packing. When boundary between solvent and H_2SiO_3 remains stationary, release pressure cautiously, pipet out most of excess solvent, and reapply pressure until ca 3 mm of solvent remains above adsorbent.

4.168 DETERMINATION

Pipet 10 ml aliquot of sample soln onto column by letting it flow slowly down inside of column without disturbing surface of the H_2SiO_3. Wash down side of column with 2 ml of the mobile solvent and force soln into column by applying 2–3 lb pressure, releasing pressure when all solvent has entered column. Add 10 ml mobile solvent and force into column. Release pressure and slowly add mobile solvent to within 3–5″ from top of column. Apply enough pressure to force solvent thru column at 3–4 ml/min. Just before last trace of dye leaves column, begin to collect 10 ml fractions, alternately using two 10 ml graduated cylinders. Transfer each fraction to 125 ml erlenmeyer and evap. to dryness, using solvent evaporator. (Evap. fractions without boiling; if boiling begins, raise flask momentarily from H_2O bath.)

Appearance of γ-isomer upon evapn is recognized by its tendency to cover bottom of flask as white residual film with typical crystal formation. When first residue of γ-isomer is recognized, begin to collect 10 ml fractions until all γ-isomer is obtained (usually no more than 8 fractions). Dissolve residue in each flask with 5 ml *n*-hexane and transfer to weighed flask, rinsing flasks successively with 5 ml portions *n*-hexane. Evap. solvent, using solvent evaporator. Evacuate flask ca 20 min. at room temp. with vac. pump. (There is little danger in evacuating 125 ml erlenmeyer; larger size erlenmeyer, however, is likely to collapse under vac.) Release vac., wipe with clean, moist towel, and let stand 5 min. Weigh, and calc. % γ-benzene hexachloride in original sample.

4.169 MELTING POINT DETERMINATION OF THE GAMMA FRACTION

Dissolve residue in min. amount of acetone and transfer quantitatively to 10 ml beaker. Evap. acetone at 40°, using filtered air stream. Scrape residue from beaker for m.p. detn. (Beaker may be set on piece of Dry Ice to insure prepn of finely powd. product.) Place material in agate mortar and mix thoroly with pestle.

Select 2 clean, dry capillary tubes and fill with sample. Be sure material is well packed into bottom of tube to insure max. contact between sample and wall of tube. Insert tubes and thermometer bulb in Thiele tube so that samples and thermometer bulb touch. Start stirrer and heater, and adjust heating rate to 1°/min. at 90°. Continue heating until sample melts or reaches 106°. Reduce heating rate to 0.5°/min. and continue heating until sample melts.

Sample m.p. is corrected temp. of bath when last solid disappears into the clear melt. If m.p. is <108°, check result by infrared method, **4.170–4.173.**

Infrared Spectrometric Method (51)—Official
(Applicable to tech. BHC)

4.170 APPARATUS

Infrared spectrometer.—With matched pair of liquid absorption cells, 0.5–1.1 mm thick.

4.171 CALIBRATION OF CELLS

Det., in spectrometer, difference between deflections of the 2 cells filled with CS_2. Plainly mark one cell to be used as sample cell for reading I. Correct values of I_0 obtained with other cell by adding or subtracting difference between cells and refer to this as cell factor F. Check factor every 10–14 days.

4.172 PREPARATION OF STANDARDS AND WORKING CURVES

Obtain α, β, γ, and δ isomers of BHC, either by fractional crystn from tech. material or as sepd materials, and recrystallize several times from solvents that have been redistd from all-glass app. Recrystallize from following solvents until m. ps. by capillary tube method become constant: α isomer from benzene followed by MeOH (m.p. ca 158°); β isomer from toluene (m.p. ca 210.5°, sealed capillary); γ isomer from MeOH (m.p. ca

113°); and δ isomer from CCl_4 followed by $CHCl_3$ (m.p. ca 138.5°).

Confirm purity of each isomer as follows: Evap. to dryness enough mother liquor from last crystn to yield at least 1 g dissolved solids, grind residue, and dry overnight in evacuated desiccator. Weigh and dissolve in enough CS_2 to make 4 g/100 ml soln. Prep. corresponding soln of the recrystd isomer as std. Compare solns of residue and std in spectrometer at wavelength points used for analysis of other isomers. Consider purity of the isomer satisfactory if absorbance of residue soln is not significantly greater than that of std at these points.

Prep. working curves of the isomers by detg transmittance of their solns in CS_2 at various concns as in **4.173.** Calc. absorbance and plot against concn in g/L.

4.173 DETERMINATION

Reduce sample of tech. BHC to ca 2 g by grinding and quartering, and dry 24 hr *in vacuo.* Weigh 1.5000 g dried material into 50 ml vol. flask and dil. to vol. with CS_2 (equiv. to 30 g/L). Shake vigorously to dissolve (β isomer is not completely sol. and will settle out). Pipet 25 ml of this sample soln into another 50 ml vol. flask and again dil. to vol. with CS_2 (equiv. to 15 g/L). Fill sample cell with the concd soln for reading *I*, and fill blank cell with CS_2, place in spectrometer, and read transmittance in duplicate at following wavelengths:

	Wavelength (microns)
Alpha	12.58
Beta	13.46
Gamma	14.53
Delta	13.22
Epsilon	13.96

Average duplicates for calcns. Repeat readings with the dil. soln (15 g/L) at α and γ wavelengths. Calc. absorbance of each of isomers at the various wavelengths from transmittance measurements by equation:

$$\text{Log}\ \frac{(F \times I_b) - (F \times I_b \times \%\ Sct)}{I_s - (F \times I_b \times \%\ Sct)} = A,$$

where F = cell factor, I_b = reading of blank cell, $\%\ Sct = \%$ scatter, I_s = reading of sample cell, and A = absorbance.

Obtain approx. concns from working curves, **4.172.** Correct absorbance at each wavelength for absorption of interfering components. (Altho β isomer has low solubility in CS_2, this isomer interferes with δ analytical point; therefore det. absorbance of β isomer in CS_2 at this point and apply as correction.) Since these new values are overcorrected, make repeated evaluations until successive values are constant, within desired precision.

Radioactive Tracer Method (52)—First Action

4.174 PRINCIPLES

Method is based on addn of pure γ isomer labeled with radioactive Cl^{36} to sample of BHC contg unknown amount of γ isomer. Detn of decrease in radioactivity from std level to dild level, on pure weighable γ fraction recovered from mixt., is measure of γ isomer content of sample. The isolation of pure γ material need not be quant.

(This analytical technique can be applied to BHC samples having wide range of γ content. Wt of unknown sample to be analyzed should be increased or decreased according to its estimated γ content, so that ratio of labeled γ added to ordinary γ in sample will approximate ratio used in this method.)

4.175 PREPARATION OF LABELED GAMMA ISOMER STANDARD

Radioactive chlorination of C_6H_6 is based on rapid establishment of exchange equilibrium between Cl and Cl-ion in aq. soln. Inactive Cl bubbled thru radioactive HCl becomes active by exchange; near-quant. transfer of radioactive Cl^{36} into Cl phase is achieved.

Place 15 ml C_6H_6 in benzene reaction tube (2, Fig. 10). By means of 4 ml pipet to which is attached hypodermic syringe, place 4 ml aq. *radioactive 0.2N HCl* contg 12 microcuries Cl^{36} in Cl-exchange tube (1, Fig. 10).

Pass ordinary *Cl from cylinder* (3, Fig. 10) 30 min. thru system up to tube contg radioactive HCl in order to displace all air from system. Open stopcock above radioactive HCl tube and bubble ordinary Cl into the HCl 6 min. at rate of 0.1 g/min. Radioactive Cl, along with excess ordinary Cl, passes into benzene reaction tube and dissolves in the benzene.

After all Cl has passed into C_6H_6, place 150 watt lamp 3″ from center of C_6H_6 tube and let it remain there until yellow-green color disappears, when reaction is complete. Introduce stream of air thru bubbler in benzene tube, apply heat to coil, and distill C_6H_6. Air stream will flush vapors thru condenser. Dry cake of ca 1 g crude labeled BHC in 75° vac. oven to remove traces of C_6H_6.

Ext. γ isomer from cake with 2 ml *n*-hexane satd with *nitromethane* by heating to b.p. and stirring vigorously. Decant ext. into 50 ml beaker and repeat extn. Wash spent cake with 2 ml cold *n*-hexane and combine with previous exts. Evap. solvent, using heat lamp placed ca 6″ above beaker, to obtain γ oil.

Add 3.5 g pure unlabeled γ isomer to γ oil. Dissolve mixt. in 16 ml alcohol (Special Denatured Formula No. 3A is satisfactory) by heating and stirring. Cool to 20° with constant stirring. Let slurry stand in bath 15 min. after crystals

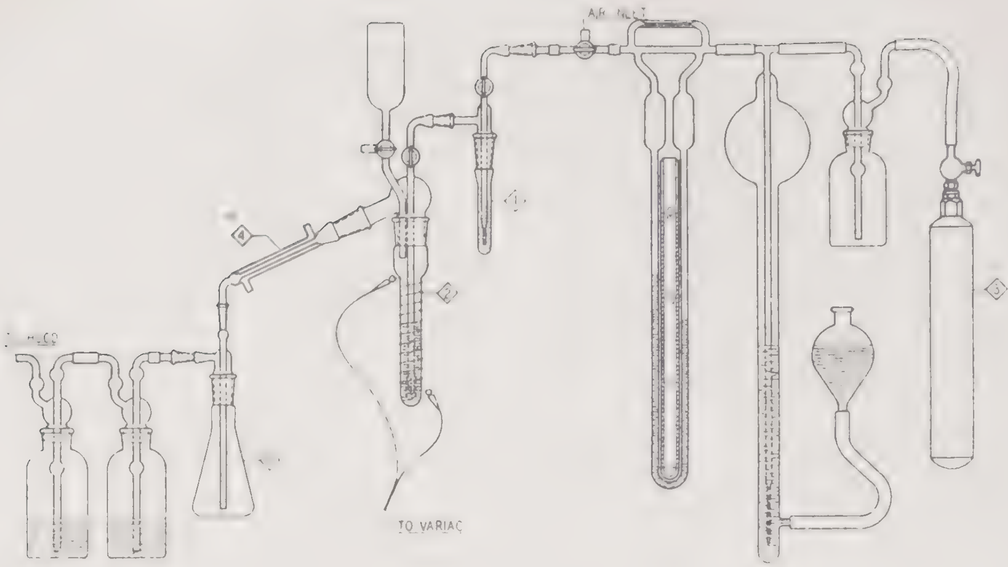

FIG. 10.—CHLORINATION APPARATUS

appear. Filter, and wash with alcohol. M.p. of dried crystals is 112.0–112.8°.

Repeat recrystn at least twice to remove all traces of isomers other than γ. Dry final crystals 3 hr at 70° in vac. oven to obtain labeled std.

4.176 ISOLATON OF GAMMA ISOMER

Weigh 120 ± 5 mg (to 0.1 mg) of labeled std into tared 15×50 mm shell vial and add 1000 ± 5 mg (to 0.1 mg) of tech. grade BHC sample.

Add 1.2 ml *perchloroethylene*, place cap (can be made from 5 ml beaker cut in half) on vial, and insert vial into well of 115° perchloroethylene heating bath (1, Fig. 11). Let vial remain in bath 15 min., stirring occasionally. (Not all of crude sample necessarily dissolves.) Cool mixt. in 20° H_2O bath (3, Fig. 11) 30 min., occasionally stirring to allow crystn of isomers other than γ. Leave cap on during crystn.

Tare 15×50 mm shell vial and place in Niederl-Niederl sulfur filtration app. (2, Fig. 11). Filter supernatant from crystals thru filter stick into tared vial. Wash crystals by adding 0.3 ml perchloroethylene and cooling to 20° while stirring. Filter wash into vial contg original filtrate.

Place vial contg filtrate and wash in evapn tube inserted in top of heating bath and evap. solvent, using air stream directed at surface of soln and adjusted to avoid splashing. Most of perchloroethylene will evap. in 30 min. Weigh residue, which is γ oil. Usual yield is 300–450 mg.

Crystallize γ oil by dissolving in 0.8 ml of *1,4-dioxane* and *n-butyl alcohol* $(1+1)$/g of mixt. while heating and stirring until soln is homogeneous. Cool 15 min. at 20° in H_2O bath; then scratch walls of vial to induce crystn. After crystals appear, let slurry stand in bath 10 more min.; then filter in Niederl-Niederl filtration app. Wash crystals with ca 5 drops cooled n-butyl alcohol. M.p. of crystals is 100–112° after drying 30 min. in 75° vac. oven.

Recrystallize material from n-butyl alcohol, using ca 4 ml solvent/g crystals, by dissolving and then cooling to 20° as before. Filter, wash with ca 3 drops n-butyl alcohol, and dry 30 min. in 75° vac. oven. Repeat recrystn, using 3 ml alcohol/g (Special Denatured Formula No. 3A alcohol is satisfactory). Dry crystals in 75° vac. oven 2 hr. Usual yield ca 50 mg; m.p. 112.0–112.8°. If m.p. is not in this range, repeat recrystn.

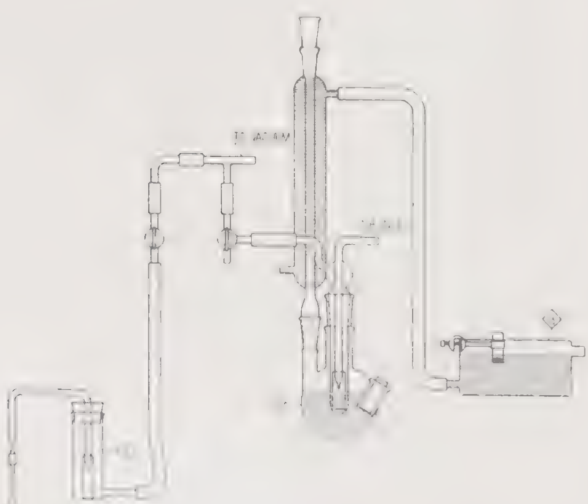

FIG. 11.—SAMPLE ISOLATION APPARATUS

4.177 COUNTING

Use thin-wall, glass, liquid-jacketed counting tube (25 mg/sq. cm, 10 ml capacity) for soln

counting. (Counting tube, Fig. 12, is available as Model J. T., N. Wood Counter Lab., 5491 Blackstone Ave., Chicago 15, Ill.)

Take background count before counting sample, filling counter tube with same solvent used to dissolve sample. Subtract this value from count of both sample and std.

Weigh, to 0.1 mg, isolated pure γ sample into 12 ml snap cap vial, add 10.0 ml acetone, and shake until sample dissolves. Transfer soln slowly into counter tube with hypodermic syringe (take care not to subject thin glass wall to too sudden pressure changes or tube will break) and count ca 15,000 total counts, noting time. Wash out tube 3 times with fresh acetone, and siphon dry. Check background again in 10 min. to make sure tube is decontaminated. Similarly prep. soln of 50 mg std and count to same approx. total count as sample, noting time.

Calc. mg γ BHC in sample, $X = A(B-C)/C$, where A = mg radioactive std added to sample; B = specific activity of std, *i.e.*, counts per min. of std/mg std counted; and C = specific activity of isolated sample, *i.e.*, counts per min. of isolated sample/mg sample counted.

NOTE.—Before radioactive compounds are made or purchased, authorization for their use must be obtained from Atomic Energy Commission, to insure that proper precaution will be observed in handling these materials.

MALATHION

Infrared Spectrophotometric Method (53)—First Action

(Applicable to dusts, dust base concentrates, and wettable powders where malathion is only active ingredient. Other extractable org. materials such as dispersing agents, emulsifiers, and solvents may interfere and should be tested for interference. S does not interfere.)

4.178 APPARATUS

Infrared spectrophotometer.—Capable of making measurements in 11–13 μ region. Both single beam and double beam instruments such as Beckman IR-5 (both as single beam and double beam instrument), Perkin-Elmer Models 21 and 112 are suitable. Use 0.5 mm cell for 4–10% dusts and 0.1 mm for 25–50% products.

4.179 REAGENTS

(a) *Malathion.*—Purified material, available from Products Laboratory, American Cyanamid Co., Princeton, N. J.

(b) *Malathion std soln.*—Accurately weigh 0.2–0.25 g purified malathion (for 4–10% dusts) or 1.2–1.25 g (for 25–50% dust base concentrates and wettable powder) into 2 oz wide-mouth bottle fitted with screw cap (vinylite liner). Add from pipet or buret 25 ml acetonitrile and shake well.

(c) *Acetonitrile.*—Essentially transparent in 11–13 μ region. (Matheson, Coleman, and Bell acetonitrile with b.p. 80–82°, has been found satisfactory.)

4.180 PREPARATION OF SAMPLE SOLUTION

Accurately weigh 5 g sample (for 4–5% dust or 25% dust base concentrate or wettable powder) or 2.5 g (for 10% dust or 50% dust base concentrate). Transfer quantitatively to 2 oz wide-mouth bottle fitted with screw cap (vinylite liner). Add from pipet or buret 25 ml acetonitrile and shake well ca 2 min. Filter thru No. 12 Whatman folded paper into g-s. flask, and stopper. (If diluent readily seps from acetonitrile, filtering may be omitted.)

4.181 DETERMINATION

Fill suitable cell, using hypodermic syringe, with appropriate std soln and obtain infrared spectra from 11.0 to 13.0 μ. (When using single beam instrument, adjust to give 80% transmittance at 11.45 μ with cell in position.) Using same instrument settings, scan sample solns in same manner.

Measure distances Y and X for both sample

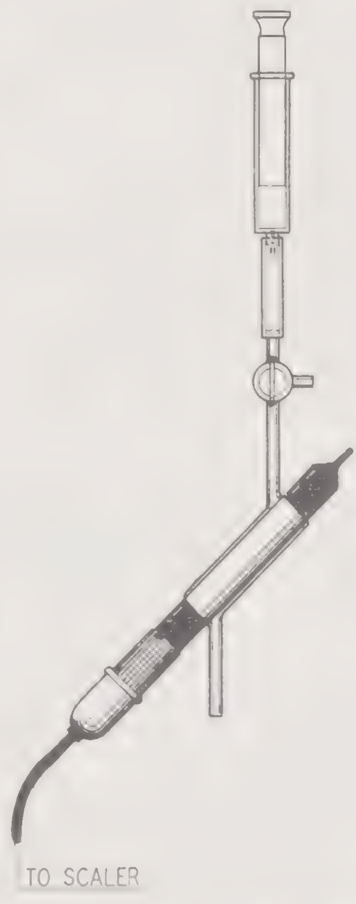

FIG. 12.—COUNTING APPARATUS

and std, where X is distance from zero line to peak at 12.2 μ, and Y is distance from zero line to base line at 11.45 μ valley. Calc. absorbance, A, of each soln as follows: Absorbance = log (Y/X).

% Malathion = (A_{sample}/A_{std})(wt std/wt sample) $\times$ % purity of std.

ORGANIC THIOCYANATES

Thiocyanate Nitrogen in Livestock or Fly Sprays (54)—Official

4.182 REAGENTS

(a) *Strong potassium polysulfide soln.*—Dissolve 180 g KOH in 120 ml H_2O. Sat. 100 ml of this soln with H_2S (ca 42 g) while cooling. Add the remaining 100 ml KOH soln and 80 g S. Shake until dissolved.

(b) *Mixed sulfide soln.*—To 100 ml (a) add 50 g $Na_2S.9H_2O$, 30 g KOH, and 200 ml H_2O.

(c) *Sodium bisulfite.*—$Na_2S_2O_5$ or $NaHSO_3$.

(d) *Copper sulfate soln.*—20% aq. soln $CuSO_4.5H_2O$.

(e) *Wash soln.*—To 300 ml H_2O add 1 ml H_2SO_4 (1+4), 1 g (c), 10 ml (d), and 12 g Na_2SO_4, and pass SO_2 into soln for 10 min.

4.183 PREPARATION OF SAMPLE

Weigh sample preferably contg ca 0.03 g thiocyanate N into 250 ml g-s. erlenmeyer. (If percentage is very low, do not unduly increase quantity of sample without correspondingly increasing quantity of the mixed sulfide soln used; 20–25 g fly spray is usually enough.) Add 35 ml of the mixed sulfide soln and shake vigorously at room temp. 10 min., during which time reaction is nearly completed. Heat to 70° on steam bath, carefully releasing pressure resulting from heating, shake 15 min. at 70°, and cool.

Removal of petroleum oil.—Transfer mixt. to separator with ca 200 ml H_2O. Add 50 ml petr. ether, shake, and drain aq. layer into 600 ml beaker. Wash petr. ether layer with two 10 ml portions H_2O, adding washings to main soln. (If emulsions form during washing, break by acidifying with H_2SO_4 (1+4).) Drain aq. layer and wash petr. ether layer with H_2O as above. Discard petr. ether layer.

4.184 DETERMINATION

Dil. combined aq. soln to ca 300 ml and neutralize with H_2SO_4 (1+4), using litmus paper as outside indicator. Add 2 ml H_2SO_4 (1+4), bring mixt. to boil quickly, and boil 8 min. to remove H_2S. Cool. If fatty acids or oils are present, transfer to separator, ext. with petr. ether, and return aq. phase to original beaker. Filter thru small büchner and transfer filtrate to beaker. Neutralize to litmus paper with 10% KOH soln and add 1 ml H_2SO_4 (1+4). Add 1 g of the Na bisulfite and stir until dissolved. Add excess (ca 15 ml) of the $CuSO_4$ soln and pass SO_2 into soln 10 min.

Let pptd CuSCN settle 2 hr, and filter with suction thru 2″ büchner coated with layer of asbestos, upon which is placed No. 42 Whatman paper (or equiv.), second layer of asbestos, layer of diatomite, and finally third layer of asbestos. If filtrate is not clear, centrifuge soln at 2000 rpm 10–15 min., and pour thru filter again. Wash filter and ppt once or twice with the wash soln, continue suction until filter pad is dry, and transfer to 800 ml Kjeldahl flask. (Filter pad may be folded in filter paper together with bits of moist filter paper used to wipe out büchner, and whole placed in the Kjeldahl flask.) Add few glass beads, 35 ml H_2SO_4, 10 g K_2SO_4, and ca 0.7 g HgO or 0.65 g Hg. Digest until colorless, then 15 min. more. Det. N as in **2.036**, second par. Perform blank analysis on paper, filter pad, and reagents.

PARATHION

Volumetric Method (55)—Official

(Applicable to technical parathion)

4.185 APPARATUS

(a) *Photoelectric colorimeter.*—Equipped with filter to give max. transmittance between 400 and 450 mμ. Spectrophotometer set at 405 mμ may also be used.

(b) *Beckman potentiometer* (*or equiv.*).—Equipped with adapter for outside Pt and calomel electrodes. Dead-stop end point equipment may also be used.

4.186 REAGENTS

(a) *Zinc dust.*—Low in Fe.

(b) *Sulfanilic acid.*—Anhyd. recrystd material. Check purity by N detn.

(c) *p-Nitrophenol.*—M. p. 112–113°.

(d) *Sodium nitrite soln.*—0.1N. Stdze weekly. Weigh accurately 0.4–0.45 g of the sulfanilic acid into 400 ml tall beaker. Add 80 ml H_2O, 10 ml HCl, 30 ml HOAc, and 5 g NaBr. Place electrodes and mechanical stirrer in reaction mixt. and titr. with the 0.1N $NaNO_2$. Add in 5 ml portions until within 1 ml of the calcd end point; then add the $NaNO_2$ soln in 0.1 ml portions until max. rise in potential is obtained. At first, 3–5 min. is required for potential to become constant; as end point is approached, especially after the 0.1 ml addns, reaction should be complete within 1 min. As alternative, dead-stop end-point technic may be used (*55*), or following spot test, adding $NaNO_2$ soln in 4 drop portions near end point: Dip glass rod into soln being titrd and touch rod quickly to piece of KI-starch paper, (e). End point is

reached when intense blue-black color appears immediately and can be obtained repeatedly during 1 min. period without further addn of $NaNO_2$.

Normality $NaNO_2$ soln = g sulfanilic acid $\times 1000$/ml $NaNO_2 \times 173.2$.

(e) *Starch iodide paper.*—Triturate 10 parts starch with 200 parts H_2O, bring to boil, and add 1 part KI. Impregnate strips of filter paper with this soln, dry, and preserve in g-s. bottles.

4.187 PREPARATION OF STANDARD CURVE OF *p*-NITROPHENOL

Weigh accurately 100 mg *p*-nitrophenol, transfer to 1 L vol. flask, and dil. to vol. with $0.1N$ NaOH. Transfer 2, 4, 6, 8, 10, and 20 ml aliquots of this soln to 100 ml vol. flasks and dil. each soln to vol. with $0.1N$ NaOH. Read absorbance of each soln in photoelec. colorimeter (400–450 mμ) or spectrophotometer (405 mμ) against H_2O as refference. Plot absorbance against concn in mg/ml.

4.188 SEPARATION OF PARATHION AND *p*-NITROPHENOL

Using weighing pipet, accurately weigh 0.6–0.9 g sample into 100 ml ether in 250 ml separator. Ext. ether soln with four (or until ext. is colorless) 20 ml portions chilled 1% Na_2CO_3 soln, collecting combined aq. layers in 200 ml vol. flask. Transfer ether layer to 400 ml tall beaker, rinsing separator with small portions of ether.

4.189 DETERMINATION OF *p*-NITROPHENOL

Add 20 ml $1N$ NaOH to combined aq. exts and dil. to vol. with H_2O. Measure absorbance of soln as in **4.187** and read concn *p*-nitrophenol in mg/ml from std curve.

$$\%\ p\text{-nitrophenol} = \frac{(\text{mg/ml}) \times 200 \times 100}{1000 \times \text{g sample}}$$

4.190 DETERMINATION OF PARATHION

Add 35 ml HOAc-HCl mixt. (9+1) to ether soln, **4.188.** Add 2 g Zn dust, cover beaker with watch glass, and heat soln gently on steam bath 45 min., or until most of ether evaps and soln is colorless. Add 30 ml HCl and heat 10 min. longer to complete soln of Zn dust. Wash down beaker and watch glass with H_2O. Filter reduced mixt. thru paper and rinse beaker thoroly with H_2O. Dil. to 125 ml and cool to room temp. Add 5 g NaBr (or KBr) and titr. with $0.1N$ $NaNO_2$ as in **4.186**(d). % parathion = ml $NaNO_2$ $\times$ normality $\times$ 29.13/g sample.

4.191 DUST PREPARATIONS AND WETTABLE POWDERS

Transfer weighed sample to thimble and ext. with 150 ml ether in Soxhlet app. 1 hr. Transfer ether ext. to 250 ml separator and sep. *p*-nitrophenol and parathion as in **4.188.** Det. sample size by parathion concn as follows: 10%, 6.75 g; 15% 4–5 g; 25%, 2.5–3.5 g.

4.192 EMULSIFIABLE CONCENTRATES

Accurately weigh sample contg 0.6–0.9 g parathion into 400 ml tall beaker. Heat on steam bath 30 min., passing gentle stream of air over surface of sample to hasten evapn. Cool to room temp., and wash into 250 ml separator with 150 ml ether. Ext. ether soln with four (or until ext. is colorless) 10 ml portions chilled 1% Na_2CO_3 soln, adding 2 g anhyd. Na_2SO_4 each time. Collect combined aq. layers in 200 ml vol. flask for detn of *p*-nitrophenol as in **4.189,** and collect ether layer in 400 ml tall beaker for detn of parathion as in **4.190.**

Colorimetric Method (56)—First Action

(Applicable to dusts and wettable powders)

4.193 APPARATUS

Photoelectric colorimeter.—As in **4.185**(a); or spectrophotometer set at 405 mμ.

4.194 PREPARATION OF *p*-NITROPHENOL STANDARD

Prep. std contg 0.3 mg *p*-nitrophenol, **4.186(c)**, and 5 ml $1N$ KOH in 50% alcohol in 100 ml 50% alcohol and measure absorbance at 405 mμ against 50% alcohol as blank. Absorbance is proportional to concn of *p*-nitrophenol. If desired, std may be prepd by using alc. soln of pure parathion and proceeding as in detn.

4.195 PREPARATION OF SAMPLE

Weigh sample contg ca 10 mg parathion and transfer to 100 ml vol. flask. Add ca 50 ml alcohol and shake occasionally 10 min. Dil. to vol. with alcohol and mix well. Filter ca 25 ml into g-s flask.

4.196 DETERMINATION OF FREE *p*-NITROPHENOL

Pipet 10 ml aliquot of above soln into 100 ml vol. flask and dil. to vol. with 50% alcohol. Add 5 drops $1N$ KOH in 50% alcohol, and measure absorbance at 405 mμ within 2 min. against 50% alcohol. Calc. free *p*-nitrophenol.

4.197 DETERMINATION OF PARATHION

Pipet 5 ml aliquot of sample soln into 200 ml round-bottom flask, and add 5 ml $1N$ KOH in 50% alcohol and few boiling chips or beads. Reflux 30 min. Cool and transfer soln to 100 ml vol. flask, using 50% alcohol. Dil. to vol. with 50% alcohol and measure absorbance at 405 mμ against 50% alcohol. If color is too dark to read, dil. aliquot with $1N$ KOH in 50% alcohol and then with 50% alcohol. Calc. parathion and correct for

free p-nitrophenol. Parathion $= 0.478 \times p$-nitrophenol.

PIPERONYL BUTOXIDE (*57*)— FIRST ACTION

4.198 APPARATUS

Photoelectric colorimeter.—Klett-Summerson, or equiv. equipped with narrow band-pass interference type filter with transmission range of ca 625–635 mμ. (Filter is available from: Baird-Atomic Inc., 33 University Road, Cambridge 38, Mass.; Bausch and Lomb Optical Co., Rochester 2, N. Y.; Farrand Optical Co., Inc., Bronx Blvd and East 238th St., New York 70, N. Y.; and Photovolt Corp., 95 Madison Ave., New York 16, N. Y.) Spectrophotometer set at wavelength in range 625–635 mμ may also be used.

4.199 REAGENTS

(a) *Purified tannic acid.*—Purify as follows: To 20 g tannic acid (at least USP grade) add 100 ml EtOAc (99%) and stir mechanically ca 1 hr. Filter by suction thru fritted glass funnel, and wash residue with three 5 ml portions EtOAc. To combined filtrate and washings add 2 g finely powdered Darco G-60 (or equiv. decolorizing C), and stir mechanically ca 0.5 hr. Filter by gravity thru double thickness of Whatman No. 1 (or equiv.) paper into graduated dropping funnel. Wash residue several times with EtOAc until vol. of filtrate and washings is ca 125 ml. Place dropping funnel over 1 L, 3-neck, round-bottom flask, equipped with mechanical stirrer, and with vigorous agitation in flask add filtrate dropwise to 5 times its vol. of toluene. Purified tannic acid is pptd immediately.

Filter by suction thru fritted glass funnel, and wash product thoroly with toluene, stirring solids with toluene to assure complete removal of EtOAc. Continue suction until practically all toluene is removed. Dry purified tannic acid in vac. oven at ca 40°, and place in tightly stoppered bottle.

(b) *Tannic acid reagent.*—Dissolve completely exactly 0.025 g purified tannic acid in 20 ml HOAc by shaking at room temp. Add 80 ml H_3PO_4 and mix thoroly. Prep. fresh daily. Store tightly stoppered as it is hygroscopic.

(c) *Purified piperonyl butoxide.*—Purify by low pressure fractional distn of technical product. Also available from Food Machinery and Chemical Corp., P.O. Box 1616, Baltimore 3, Md.

(d) *Piperonyl butoxide std soln.*—Weigh exactly 1.0000 g purified piperonyl butoxide into 100 ml vol. flask. (Hypodermic syringe and needle are convenient for adding sample to flask.) Dil. to mark with deodorized kerosene and mix well. Pipet 10 ml of this soln into 200 ml vol. flask. Dil. to mark with deodorized kerosene and mix well. This soln contains 50 mmg piperonyl butoxide /0.1 ml and is stable for several months. If std is to be used with sample contg pyrethrum, add to std before initial diln enough pyrethrum ext. to give ratio piperonyl butoxide to pyrethrins similar to sample.

4.200 PREPARATION OF SAMPLE

Weigh accurately sample contg 0.5–1.5 g piperonyl butoxide into tared 100 ml vol. flask, dil. to mark with deodorized kerosene, and mix well. Pipet 10 ml of this soln into 200 ml vol. flask, dil. to mark with deodorized kerosene, and mix well.

4.201 DETERMINATION

Pipet 0.1 ml (from 1 ml pipet graduated in 0.1 ml) of sample soln into 18×150 mm test tube. Add exactly 5 ml tannic acid reagent and shake tube vigorously 1 min. Treat std and blank, consisting of 0.1 ml of deodorized kerosene, simultaneously in same manner.

Place test tubes in test-tube basket and place in vigorously boiling H_2O bath 5 min. Remove basket and let tubes cool to room temp. Transfer solns to colorimeter tubes and read, against H_2O, using 625–635 mμ filter or setting. (After cooling to room temp. there is no appreciable change in color value for several hr.)

Subtract absorbance reading of deodorized kerosene from readings of both sample and std.

Mg piperonyl butoxide $= A_{sample} \times 0.05 / A_{std}$.

SABADILLA ALKALOIDS (*58*)— FIRST ACTION

(In dust formulations)

4.202 DETERMINATION

Weigh 10 g mixed 50% sabadilla dust (or corresponding quantity of lesser concn) into 500 ml g-s. erlenmeyer. Add exactly 300 ml ether-$CHCl_3$ (3+1), and shake 5 min. Make alk. with 10 ml NH_4OH and shake 2 hr on shaking machine. Let stand overnight; then shake 1 hr.

Filter, avoiding evapn. Place 200 ml aliquot in 500 ml separator, acidify with H_2SO_4 (3+97), and shake; withdraw small amount of aq. layer and test with litmus paper, returning soln to separator. Add 50 ml of the dil. H_2SO_4 and shake. Let sep. and transfer acid ext. to second 500 ml separator. Add 50 ml petr. ether to acid ext. and shake. Let layers sep. and transfer acid ext. to third separator. Repeat extn of soln in first separator with two 50 ml portions of the dil. H_2SO_4, using same 50 ml petr. ether in second separator for washing. Collect acid exts in third separator.

Make acid exts alk. to phthln with NH_4OH. Ext. with three 50 ml portions $CHCl_3$. Wash

each $CHCl_3$ ext. by shaking gently with same 100 ml portion H_2O in fourth separator. (If emulsion forms, add small amount of anhyd. Na_2SO_4.)

Filter each $CHCl_3$ ext. thru cotton into weighed 250 ml flask. Evap. $CHCl_3$ on steam bath. Add few ml alcohol, and evap. again. Dry 1 hr at 100° and weigh sabadilla alkaloids. Calc. % total alkaloids.

4.203 QUALITATIVE TEST

Add 1–2 ml H_2SO_4 to few mg of residue, **4.202.** Presence of sabadilla alkaloids is indicated by yellow color that gradually becomes intensely red with greenish fluorescence.

TETRAETHYLPYROPHOSPHATE (TEPP) (*59*)—OFFICIAL

4.204 REAGENTS

(**a**) *Indicator.*—0.1% aq. soln Me red or chlorophenol red.

(**b**) *Amberlite IR-4B(OH) (free base form) resin.*—Analytical grade. Amberlite IR-45, Dowex 3, or equiv. are satisfactory.

4.205 PREPARATION OF RESIN COLUMN

Screen resin to remove particles <30-mesh. Slurry 30 g screened resin with H_2O, and pour into 100 ml buret contg small plug of glass wool at bottom. Wash resin column with 150 ml 3% NaOH soln at flow rate of ca 5 ml/min. and then rinse with H_2O until effluent is acid to phthln, adjusting stopcock of buret so flow rate is ca 25 ml/min. Wash with aq. acetone (1+3) to displace H_2O. Column is now ready for use.

NOTES: Because channeling may result if column runs dry, keep liquid level ca 1″ above resin bed at all times. Because resin tends to pack in column as it adsorbs acidic material, expand resin bed after each detn before adding new sample by back-washing with acetone (1+3) as follows: Connect large funnel to tip of buret with rubber hose, and add the dil. acetone from funnel until liquid level reaches top of buret; let resin settle, and then let soln flow from buret until surface is 1″ above resin bed. Column is now ready to receive next sample.

After 8–10 samples have passed thru column, regenerate resin by repeating original treatment with 3% NaOH soln, H_2O, and acetone (1+3). Washing with the dil. acetone must be continued until effluent is colorless.

4.206 DETERMINATION

(**a**) *In purified or technical grades of tetraethylpyrophosphate not mixed with solvent, emulsifying agent, etc.*—From 5–10 ml weighing buret weigh by difference, to nearest mg, 2.5 g sample (1.0 g if tetraethylpyrophosphate content is >50%) into 50 ml acetone (1+3) in 125 ml separator. Mix by swirling, and let soln stand 15 min. at 25±2°. Run soln thru resin column by gravity at ca 25 ml/min., and catch effluent in 250 ml vol. flask. Wash separator and column with three 50 ml portions acetone (1+3), collecting washings in same flask. Dil. combined effluent to vol. with H_2O, mix, and transfer 100 ml aliquot to 250 ml beaker. Add 50 ml 0.1*N* NaOH to contents of beaker, stir well, let stand 30 min. at room temp., and back-titr. with 0.1*N* HCl, using pH meter (or indicator, **4.204**(**a**), if pH meter is not available). Calc. % tetraethylpyrophosphate as follows:

% tetraethylpyrophosphate

$= \text{Net ml } 0.1N \text{ NaOH} \times 3.67/\text{wt sample taken.}$

(**b**) *In formulations of tetraethylpyrophosphate containing organic solvent and emulsifying agent.*—Proceed as in (**a**), except filter the acetone soln thru 1″ cotton plug in cylindrical funnel (1″ diam., 3″ long) before adding it to column if oil seps from the soln. Pass the acetone washings successively thru separator, cylindrical funnel, and resin column. (Cotton plug absorbs the oil.)

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5. Disinfectants

Phenol Coefficient (*1*)—Official

(Applicable to testing disinfectants miscible with H_2O that do not exert bacteriostatic effects that cannot be neutralized by one of 3 subculture media specified, or overcome by suitable subtransfer procedures. The 95% confidence limits are ±12%.)

1. Using Salmonella typhosa

5.001 REAGENTS

(a) *Culture media.*—(1) *Nutrient broth.*—Boil 5 g beef ext. (Difco), 5 g NaCl, and 10 g peptone (Armour, quality specially prepd for disinfectant testing) or USP peptic digest of animal tissue (bacteriological peptone) in 1 L H_2O 20 min., and dil. to vol. with H_2O; adjust to pH 6.8. (If colorimetric method is used, adjust broth to give dark green color with bromothymol blue.) Filter thru paper, place 10 ml quantities in 20×150 mm bacteriological test tubes, plug with cotton, and sterilize at 15 lbs pressure 20 min. Use this broth for daily transfers of test cultures.

(2) *Nutrient agar.*—Dissolve 1.5% Bacto agar (Difco) in nutrient broth and adjust to pH 7.2–7.4 (blue-green with bromothymol blue), tube, plug with cotton, sterilize, and slant.

(3) *Subculture media.*—Use (a), (b), or (c), whichever gives lowest result:

(a) *Nutrient broth* described in **(a)**(1);

(b) *Fluid thioglycollate medium USP XVI:* Mix 0.5 g L-cystine, 0.75 g agar, 2.5 g NaCl, 5.5 g dextrose, 5.0 g H_2O-sol. yeast ext., and 15.0 g pancreatic digest of casein with 1 L H_2O; heat on H_2O bath to dissolve, add 0.5 g Na thioglycollate or 0.3 ml thioglycollic acid, and adjust with 1*N* NaOH to pH 7.0±0.1; reheat without boiling and filter thru moistened filter paper; add 1.0 ml freshly prepd 0.1% Na resazurin soln; transfer 10 ml quantities to 20×150 mm bacteriological test tubes, plug with cotton, and sterilize at 15 lbs pressure 20 min.; cool at once to 25° and store at 20–30°;

(c) "*Letheen broth*": Dissolve 0.7 g lecithin (Azolectin) and 5.0 g sorbitan monoöleate ("Tween 80") in 400 ml hot H_2O and boil until clear; add 600 ml soln of 5.0 g beef ext. (Difco), 10.0 g peptone (Armour) or 10 g USP peptic digest of animal tissue, and 5 g NaCl in H_2O, and boil 10 min.; adjust with 1*N* NaOH and/or 1*N* HCl to pH 7.0±0.2 and filter thru coarse paper; transfer 10 ml quantities to 20×150 mm bacteriological test tubes, plug with cotton, and sterilize at 15 lbs pressure 20 min. With oxidizing products and products formulated with toxic compounds contg certain heavy metals like Hg, (b) will usually give lowest result. With products contg cationic surface active materials, (c) will usually give lowest results.

(b) *Test organism.*—Hopkins strain 26 of *Salmonella typhosa* (Zopf) Weldin, FDA, ATCC No. 6539 (formerly called *Bac. typhosus* and *Eberthella typhosa*). Maintain stock culture on nutrient agar slants by monthly transfers. Incubate new stock transfer 2 days at 37°; then store at 2–5°. From the stock culture inoculate tube of nutrient broth and make at least 4 consecutive daily transfers (not >30) in nutrient broth, incubating at 37°, before using culture for testing (if only 1 daily transfer has been missed it is not necessary to repeat the 4 consecutive transfers). Use 22–26 hr culture of organism grown in nutrient broth at 37° in test. Shake, and let settle 15 min. before using.

(c) *Phenol.*—Use phenol, USP, that congeals at 40° or above. Use 5% soln as stock soln and keep in well-stoppered amber bottles in cool place, protected from light. Stdze with 0.1*N* K or Na bromide-bromate soln, **32.128**.

5.002 APPARATUS

(a) *Glassware.*—1, 5, and 10 ml volumetric pipets; 1, 5, and 10 ml Mohr pipets graduated to 0.1 ml or less; 100 ml g-s. cylinders graduated in 1 ml divisions; Pyrex lipped test tubes, 25×150 mm (medication tubes); lipless bacteriological test tubes 20×150 mm (test culture and subculture tubes). Plug medication tubes with cotton wrapped in 1 layer of cheese cloth. Sterilize all glassware 2 hr in hot air oven at 180°. Loosely plug pipets with cotton at mouth and place in closed metal containers before sterilizing.

(b) *Water bath.*—Insulated, relatively deep H_2O bath with cover having at least 10 well-spaced holes which admit medication tubes but not their lips.

(c) *Racks.*—Any convenient style. Blocks of wood (size depending on space in incubator) with deep holes are satisfactory. Have holes well spaced to insure quick manipulation of tubes. It is convenient to have them large enough to admit medication tubes while dilns are being made.

(d) *Transfer loop.*—Make 4 mm i.d. single loop at end of 2–3″ Pt or Pt alloy wire No. 23 B & S

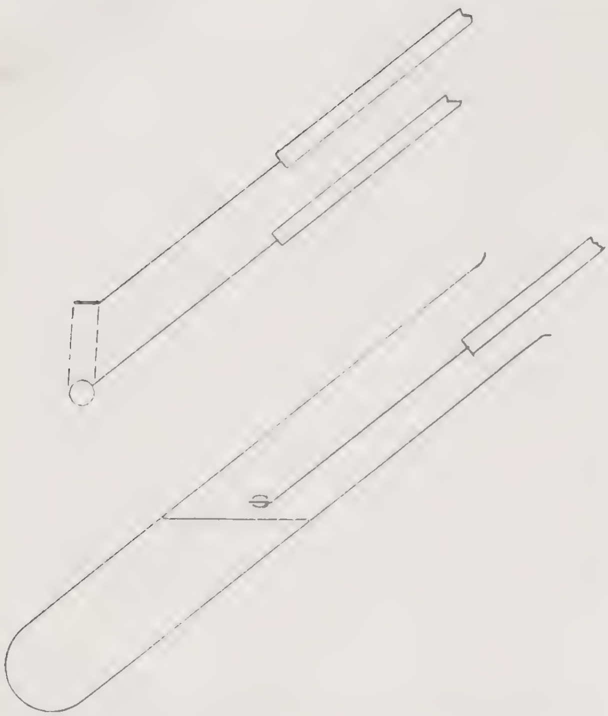

FIG. 13.—TRANSFER LOOP AND MANNER OF USING IN PHENOL COEFFICIENT TECHNIC

gauge. Fit other end in suitable holder (glass or Al rod). Bend loop at 30° angle with stem, Fig. 13.

5.003 OPERATING TECHNIC

Make 1% stock diln of substance to be tested (or any other convenient diln, depending on anticipated concn) in g-s. cylinder. Make final dilns, from the 1% stock diln, directly into medication tubes and remove all excess >5 ml. (Range of dilns should cover killing limits of disinfectant in 5–15 min. and should at same time be close enough for accuracy.) From the 5% stock phenol soln make 1–90 and 1–100 dilns directly into medication tubes. Place these tubes, contg 5 ml each of final dilns of disinfectant and of phenol, and tube contg test culture in H_2O bath at 20° and leave 5 min. Add 0.5 ml of the test culture to each of dilns at time intervals corresponding to intervals at which transfers are to be made. (Thus, by time 10 tubes have been seeded at 30 sec. intervals, 4.5 min. has elapsed, and 30 sec. interval intervenes before transference to subcultures begins.) Add culture from graduated pipet large enough to seed all tubes in any one set.

In inoculating medication tubes, hold them in slanting position after removal from bath, insert pipet to just above surface of disinfectant, and run in culture without letting tip touch disinfectant. After adding culture, agitate tubes gently but thoroly to insure even distribution of bacteria, and replace in bath; 5 min. after seeding first medication tube, transfer 1 loopful of mixt. of culture and dild disinfectant from medication tube to corresponding subculture tube. To facilitate transfer of uniform drops of medication mixt., hold tube at 60° angle, and withdraw loop so that plane of loop is parallel with surface of liquid (Fig. 13). After 30 sec., transfer loopful from second medication tube to second subculture tube and continue process for each successive diln; 5 min. after making first transfer begin second set of transfers for 10 min. period, and finally repeat for 15 min. period.

Gently agitate medication tubes before taking each interval loop subsample for transfer to subculture medium. Before each transfer heat loop to redness in flame and flame mouth of every tube. Sterilize loop immediately after each transfer (before replugging tubes) to allow time for cooling. Use care in transferring and seeding to prevent pipet or needle from touching sides or mouth of medication tube, and see that no cotton threads adhere to inner sides or mouths of tubes. Incubate subcultures 48 hr at 37° and read results. Thoroly agitate individual subculture tubes before incubation. Macroscopic examination is usually sufficient. Occasionally 3 day incubation period, agar streak, microscopic examination, or agglutination with antityphoid serum may be necessary to det. feeble growth or suspected contamination.

5.004 CALCULATION

Express results in terms of phenol coefficient number, or highest diln killing test organism in 10 min. but not in 5 min., whichever most accurately reflects germicidal value of disinfectant. Phenol coefficient is number obtained by dividing numerical value of greatest diln (denominator of fraction expressing diln) of disinfectant capable of killing *S. typhosa* in 10 min. but not in 5 min. by greatest diln of phenol showing same results.

Example:

DILN	5 MIN.	10 MIN.	15 MIN.
Disinfectant (X):			
1–300	0	0	0
1–325	+	0	0
1–350	+	0	0
1–375	+	+	0
1–400	+	+	+
Phenol:			
1– 90	+	0	0
1–100	+	+	+

Phenol coefficient would be $\frac{350}{90} = 3.89$.

Test is satisfactory only when phenol control gives one of following readings:

PHENOL	5 MIN.	10 MIN.	15 MIN.
1– 90	+ or 0	+ or 0	0
1–100	+	+	+ or 0

If none of dilns of disinfectant shows growth in 5 min. and killing in 10 min., estimate hypothetical diln only when any 3 consecutive dilns show following results: first, no growth in 5 min.; second, growth in 5 and 10 min. but not in 15 min.; and third, growth in 5, 10, and 15 min.

Example:

Disinfectant (X):

DILN	5 MIN.	10 MIN.	15 MIN.
1–300	0	0	0
1–350	+	+	0
1–400	+	+	+

Phenol:

1– 90	0	0	0
1–100	+	+	0

Phenol coefficient would be $\frac{325}{95} = 3.42$.

To avoid giving impression of fictitious accuracy, calc. phenol coefficient to nearest 0.1. Thus, in examples cited above, phenol coefficients would be reported as 3.9 and 3.4, instead of 3.89 and 3.42.

NOTE: It is commonly accepted criterion that disinfectants for general use be at diln equiv. in germicidal efficiency to 5% phenol against *S. typhosa* thru use of calcn 20×*S. typhosa* coefficient to det. no. of parts H_2O in which 1 part of germicide should be incorporated; however, this should be regarded as highest possible diln which could be considered for practical disinfection and is subject to confirmation by Use-Diln Method. Where this criterion is found invalid, use highest diln that will kill *S. choleraesuis* in Use-Diln Method as index to highest diln for use in practical disinfection.

5.005 *2. Using Staphylococcus aureus (1)*

Proceed as in **5.001–5.004,** except to change phenol dilns and test organisms. Use temp. of 20°. Use 22–26 hr culture of *Staph. aureus* FDA 209, ATCC No. 6538, having at 20° at least resistance indicated by following:

PHENOL	5 MIN.	10 MIN.	15 MIN.
1–60	+	0	0
1–70	+	+	+

NOTE: Calc. results as in **5.004.** If conversion 20×*Staph. aureus* coefficient is used to det. no. of parts of H_2O in which 1 part of germicide may be incorporated to disinfect where pyogenic organisms are the objective, this diln is subject to confirmation by Use-Diln Method. Where this criterion is found invalid, use highest diln that will kill both *Staph. aureus* and *S. choleraesuis* in Use-Diln Method as index to highest diln for use in practical disinfection in hospitals, clinics, and other places where pyogenic bacteria may have special significance.

Use-Dilution Method (*2*)—Official

(Applicable to testing disinfectants miscible with H_2O to confirm phenol coefficient results and to det. max. dilns effective for practical disinfection.)

1. Using Salmonella choleraesuis

5.006 REAGENTS

(**a**) *Culture media.—See* **5.001**(**a**).

(**b**) *Test organism, Salmonella choleraesuis.*—(ATCC 10708). Maintain stock culture on nutrient agar slants by monthly transfers. Incubate new stock transfer 2 days at 37°; then store at 2–5°. From stock culture inoculate tube of nutrient broth and incubate at 37°. Make 3 consecutive 24 hr transfers; then inoculate tubes of nutrient broth (2 for each 10 carriers to be tested), using one loop of inoculum with each tube; incubate 48–54 hr at 37°.

(**c**) *Phenol.—See* **5.001**(**c**).

(**d**) *Sterile distilled water.*—Prep. stock supply H_2O in 1 L flasks, plug with cotton, sterilize at 15 lb pressure 20 min., and use to prep. dilns of medicants.

(**e**) *Asparagine soln.*—Make stock supply of 0.1% asparagine ("Bacto") soln in H_2O in erlenmeyer of convenient size, plug with cotton, and sterilize at 15 lb pressure 20 min. Use to cover metal carriers for sterilization and storage.

(**f**) *Sodium hydroxide soln.*—Approx. 1*N* (4%). (For cleaning metal carriers before use.)

5.007 APPARATUS

(**a**) *Glassware.*—As in **5.002**(**a**). Also: straight side Pyrex test tubes, 20×150 mm; 15×110 mm petri dishes; 100 ml, 300 ml, and 1 L erlenmeyers. Sterilize petri dishes in closed metal containers.

(**b**) *Water bath and racks.—See* **5.002**(**b**) and (**c**).

(**c**) *Transfer loops and needles.*—(1) *See* **5.002**(**d**). (2) Make 3 mm right angle bend at end of 2–3″ nichrome wire No. 18 B&S gauge. Have other end in suitable holder (glass or Al rod).

(**d**) *Carriers.*—Polished stainless steel cylinders (penicillin cups), 8±1 mm o.d., 6±1 mm i.d., length 10±1 mm, of type 304 stainless steel, SS 18–8. (Obtainable from S. & L. Metal Products Corp., 25 Lafayette St., Brooklyn 1, N.Y.)

(**e**) *Petri dishes.*—Have available ca 6 sterile petri dishes matted with layer of S&S No. 597, 9 cm filter paper.

5.008 OPERATING TECHNIC

Soak ring carriers overnight in 1*N* NaOH, rinse with tap H_2O until rinse H_2O is neutral to phthln, then rinse twice with distd H_2O; place cleaned ring carriers in multiples of 10 in cotton plugged erlenmeyers or 25×150 mm cotton plugged Pyrex test tubes, cover with asparagine soln, **5.006**(**e**), sterilize at 15 lb 20 min., cool, and hold at room temp. Transfer 20 sterile ring carriers, using flamed nichrome wire hook, into 20 ml 48–54 hr nutrient broth test culture in sterile 25×150

mm medication tube. After 15 min. contact period remove cylinders, using flamed nichrome wire hook, and place on end in vertical position in sterile petri dish matted with filter paper, **5.007**(e). Cover and place in incubator at 37° and let dry not <20 min. and not >60 min. Hold broth culture for detn of its resistance to phenol by phenol coefficient method, **5.003.**

From 5% stock phenol soln make 1–90 and 1–100 dilns directly into medication tubes. Place tube for each diln in H_2O bath and let come to 20°. Make stock soln of the germicide to be tested in sterile g-s. cylinder. From this soln make 10 ml dilns to be tested, depending upon phenol coefficient found and/or claimed against *S. typhosa* at 20°, directly into each of ten 25×150 mm medication tubes, and then place the 10 tubes in H_2O bath at 20° and let come to temp. Det. diln to be tested by multiplying phenol coefficient number found and/or claimed by 20 to det. number of parts H_2O in which one part germicide is to be incorporated.

Add 0.5 ml of the test culture suspension to the 1–90 diln of the phenol control; after 30 sec. interval, add 0.5 ml to the 1–100 diln of the control, using sterile cotton plugged pipets. After adding culture, agitate tubes gently but thoroly to distribute bacteria evenly, and replace in bath; 5 min. after seeding first medication tube, transfer 1 loopful of mixt. of culture and dild phenol from medication tube to corresponding subculture tube. After 30 sec. interval, transfer loopful from second medication tube; 5 min. after making first set of transfers begin second set of transfers for 10 min. period; and finally repeat for 15 min. period. Use technic of loop sampling, flaming loop and mouths of tubes, and agitating medication and subculture tubes as described in phenol coefficient method, **5.003.** Incubate subcultures 48 hr at 37° and read results. Resistance in 48–54 hr culture of *S. choleraesuis* should fall within range specified for 24 hr culture of *S. typhosa* in phenol coefficient method.

Add 1 contaminated dried cylinder carrier at 1 min. intervals to each of the 10 tubes of the use-diln of germicide to be tested. Thus, by time the 10 tubes have been seeded, 9 min. will have elapsed, plus 1 min. interval before transfer of first carrier in series to individual tube of subculture broth. This interval is constant for each tube with prescribed exposure period of 10 min. The 1 min. interval between transfers allows adequate time for flaming and cooling nichrome wire hook and making transfer in manner so as to drain all excess medication from carrier. Flame lips of medication and subculture tubes in conventional manner. Immediately after placing carrier in medication tube, swirl tube 3 times before placing it back in bath. Shake subculture tubes thoroly, incubate 48 hr at 37°, and report results as + (growth) or − (no growth) values.

Where there is reason to suspect that lack of growth at conclusion of incubation period may be due to bacteriostatic action of medicant adsorbed on carrier that has not been neutralized by subculture medium employed, transfer each ring to new tube of sterile medium and reincubate for addnl period of 48 hr at 37°. Where soln under test is such that material adsorbed on ring carriers and transferred into subculture medium makes it unsuitable for growth of test organism, as may be case with concd acids and alkalies, products carrying antibiotics, and wax emulsions, transfer each ring to new tube of sterile medium 30 min. after initial transfer and incubate both primary and secondary subculture tubes 48 hr at 37°. Results showing no growth on all 10 carriers will confirm phenol coefficient number found. Results showing growth on any of the 10 carriers indicate phenol coefficient number to be unsafe guide to diln for use. In latter case, repeat test, using lower dilns (higher concns) of germicide under study. Max. diln of germicide which kills test organism on the 10 carriers in 10 min. interval represents max. safe use-diln for practical disinfection.

5.009 *2. Using Staphylococcus aureus*

Proceed as in **5.008** except change phenol dilns to those specified **5.005** and test organism. Use 48–54 hr culture of *Staph. aureus* FDA 209, ATCC No. 6538 having at least the resistance specified for 24 hr culture at 20° in phenol coefficient method, **5.005.** Results showing growth on any of the 10 carriers indicate that diln is too high for use in disinfecting where pyogenic bacteria must be killed. In such cases repeat test, using lower dilns (higher concns). Max. diln of germicide which kills both this test organism and *S. choleraesuis* on the 10 carriers in 10 min. interval represents max. safe use-diln for disinfecting in hospitals, clinics, and other places where pyogenic bacteria have special significance.

NOTE: While killing in 10 of 10 replicates specified provides reasonably reliable index in most cases, killing in 50 out of 50 replicates is necessary for confidence level of 95%.

Available Chlorine Germicidal Equivalent Concentration (*3*)—Official

(Applicable to water-miscible disinfectants for detg available Cl germicidal equiv. concns with products offered for use as germicidal rinses for previously cleaned nonporous surfaces, especially where speed of action and capacity are essential considerations.)

5.010 REAGENTS

Use reagents specified in **5.001**, and in addn:

(**a**) *Sterile distilled H_2O.*—*See* **5.006**(**d**).

(**b**) *Sterile phosphate buffer soln.*—pH 8.0. Add 97.5 ml soln contg 11.61 g anhyd. K_2HPO_4 in 1 L H_2O to 2.5 ml soln contg 9.08 g anhyd. KH_2PO_4 in 1 L H_2O and sterilize 20 min. at 15 lbs in cotton plugged erlenmeyer.

(**c**) *NaOCl std stock soln.*—Approx. 5%. Store NaOCl stock soln in tightly closed bottle in refrigerator, and det. exact available Cl concn at frequent intervals by As_2O_3 titrn, **4.139.**

(**d**) *Test organisms.*—Use *S. typhosa* ATCC No. 6539 or *Staph. aureus* ATCC No. 6538 or both.

5.011 APPARATUS—*See* **5.002.**

GERMICIDE	CONCN PPM AVAIL. Cl	SUBCULTURE SERIES									
		1	2	3	4	5	6	7	8	9	10
NaOCl control	200	−	−	−	−	−	+	+	+	+	+
	100	−	−	−	+	+	+	+	+	+	+
	50	−	−	+	+	+	+	+	+	+	+
Unknown (X)	25	−	−	−	−	−	+	+	+	+	+
	20	−	−	−	−	+	+	+	+	+	+
	10	−	+	+	+	+	+	+	+	+	+

− = No growth   + = growth

5.012 OPERATING TECHNIC

Det. resistance of test culture to phenol as in **5.001–5.005**, and use cultures with resistance specified. Prep., in sterile g-s. cylinders, NaOCl solns contg 200, 100, and 50 ppm available Cl in the sterile buffer soln, **5.010**(**b**). Transfer 10 ml of each soln to 25×150 mm medication tubes, place tubes in 20° H_2O bath, and let come to temp.

Starting with tube contg 200 ppm available Cl, add 0.05 ml test culture prepd as in **5.001**(**b**), shake, and return to H_2O bath. After 1 min. make transfer to tube of appropriate subculture medium, **5.001**(**a**)(3), using flamed 4 mm loop. At 1.5 min. add another 0.05 ml culture to the 200 ppm Cl soln, shake, and return to bath. After addnl 1 min. interval (2.5 min. in test) make second subculture in same manner, and in 30 sec., or at 3 min. time in test, add another 0.05 ml culture, shaking and returning to H_2O bath. After another 1 min. interval (4 min. in test) make another transfer to tube of subculture medium.

Repeat this procedure to give total of 10 added increments. This will require total time of 14.5 min. for each soln and addn of 0.5 ml of total culture with subculture at std 1 min. intervals after addn of culture aliquots. At conclusion of test shake all subculture tubes and incubate 48 hr at 37°.

Repeat this procedure with solns contg 100 and 50 ppm of available Cl. Prep. soln of germicide to be tested at concn recommended or selected for study in sterile H_2O in g-s. graduate. Transfer 10 ml to 25×150 mm medication tubes, place in H_2O bath, and let come to temp. Repeat procedure with this soln.

To be considered equiv. in disinfecting activity to 200 ppm available Cl, unknown germicide must show absence of growth in as many consecutive tubes of subculture tube series as the 200 ppm available Cl std. Det. activity equiv. to 100 and 50 ppm available Cl in same manner.

Example:

From above result, 25 ppm soln of germicide X could be considered equiv. to 200 ppm soln of available Cl, and 20 ppm soln equiv. to 100 ppm of available Cl, but 10 ppm soln of germicide X would not be considered equiv. in germicidal activity to 50 ppm of available Cl.

Draw conclusions relative to germicidal equiv. concns only when resistance of test culture to NaOCl control is such that least 1 negative increment is obtained at 50 ppm concn and 1 positive increment is obtained at 200 ppm level.

Sporicidal Test (*4*)—Official

(Applicable to germicides for detg presence or absence of sporicidal activity and potential effectiveness in disinfecting against specified spore forming bacteria in various situations.)

5.013 REAGENTS

(a) *Culture media.*—(1) *Soil extract nutrient broth.*—Ext. 1 lb garden soil in 1 L H_2O, filter several times thru S&S No. 588 paper, and dil. to vol. (pH should be 5.2 or higher.) Add 5 g beef extract (Difco), 5 g NaCl, and 10 g peptone (Armour), boil 20 min., adjust with 1*N* NaOH to pH 6.9, filter thru paper, dil. to vol., dispense into 20×150 mm test tubes, plug with cotton, and

sterilize 20 min. at 15 lbs pressure. Use this broth to propagate test culture of *Bacilli*.

(2) *Nutrient agar.—See* **5.001(a)**(2). Use slants of this medium to maintain stock culture of *Bacilli*.

(3) *Modified fluid thioglycollate medium USP XVI.*—Prep. as in **5.001(a)**(3)(b), except add 20 ml 1N NaOH to each liter before dispensing for sterilization. Use this medium to subculture spores exposed to 2.5N HCl. For spores exposed to unknown germicides use fluid thioglycollate medium USP XVI.

(4) *Soil extract-meat-egg medium.*—Add 1.5 g Bacto Egg-Meat Medium dehydrated (Difco) to 20×150 mm test tubes; then add 10 ml garden soil extract, (1), plug with cotton, and sterilize 20 min. at 15 lbs pressure. Use this medium to propagate test cultures of *Clostridia* and maintain stock cultures of species of this genus.

(**b**) *Test organisms.*—Any species of *Clostridia* or *Bacilli*. Strains of *Bacillus subtilis* and *Clostridium sporogenes* may be employed in routine evaluations, but method is applicable for use with strains of *B. anthracis, Cl. tetani*, or other species.

(**c**) *Dilute hydrochloric acid.*—2.50N. Use to det. resistance of the dried spores. Stdze and adjust to 2.50N as in **42.010**.

5.014 APPARATUS

(**a**) *Glassware.*—Pyrex lipped test tubes 25 ×150 mm; 100 ml g-s. cylinders graduated in 1 ml divisions; supply of 15×110 mm petri dishes matted with 2 sheets S&S No. 597, 9 cm filter paper. Sterilize all glassware 2 hr in air oven at 180°C.

(**b**) *Water bath.—See* **5.002(b)**.

(**c**) *Racks.—See* **5.002(c)**.

(**d**) *Transfer loop and hook.—See* **5.007(c)**.

(**e**) *Suture loop carrier.*—From spool of size 3 surgical silk suture, prep. std loops by wrapping the silk around ordinary pencil 3 times, slipping coil so formed off end of pencil, and holding it firmly with thumb and index finger while passing another piece of suture through coil, knotting and tying it securely. Then shear off end of coil and knotting suture to within $\frac{1}{16}''$. This should provide over-all length of ca $2\frac{1}{2}''$ of suture in 2-loop coil that can be conveniently handled in ordinary aseptic transfer procedures.

Ext. loops in groups of 20 by immersion in 10 ml petr. ether in stoppered lipped test tube, shaking frequently during 30 min. at room temp. and hold overnight (18–24 hr) at 2–5°. Shake, remove loops, drain, and dry.

(**f**) *Cylinder carriers.*—"Pennycylinders" porcelain, 8±1 mm o.d., 6±1 mm i.d., 10±1 mm long. (Available from Fisher Scientific Co., catalog No. 7-907.)

5.015 OPERATING TECHNIC

Grow all *Bacilli* in soil ext. nutrient broth and all *Clostridia* in soil ext. meat-egg medium. Inoculate tubes, using 1 loop of the stock culture, and incubate 72 hr at 37°. Place supply of suture loop and cylinder carriers in sep. petri dishes matted with filter paper, and sterilize 20 min. at 15 lbs. Use new loops for each test. Place 5 sterile loops or 5 sterile cylinders in each 72 hr culture, agitate vigorously, and let stand 15 min. Withdraw loops, or cylinders, place in sterile matted petri dishes, and let dry 22–26 hr at room temp. Make all suture loop and cylinder transfers with 2–3″ nichrome wire hook, flamed between transfers.

Transfer 10 ml 2.5N HCl, **5.013(c)**, into sterile cotton plugged 25×150 mm lipped test tube. Place tube in 20° constant temp. H_2O bath and let come to temp. Transfer rapidly 4 dried, contaminated loop or cylinder carriers to acid tube. Transfer remaining dried, contaminated suture loop or cylinder carriers to tube of the thioglycollate subculture medium, **5.013(a)**(3), as viability control. After 5, 10, 20, and 30 min., withdraw individual loops or cylinders from the acid and transfer to individual tubes of thioglycollate subculture medium. Rotate each tube vigorously 20 sec., and incubate 1 week at 37°.

Reliable readings can usually, but not always, be made after 48 hr incubation. If it appears that pH of subculture medium has been reduced by carried-over acid to level below that which will permit growth of test organism, transfer loops or cylinders to fresh tubes of medium and reincubate for second 7 day period. Test spores should resist the HCl at least 2 min., and many will resist the HCl full 30 min. period and longer.

If this test shows that resistant dried spores are present (vegetative cells will not show measurable resistance against 2.5N HCl), use duplicate lots of dried, contaminated suture loops or cylinders in lots of 5, drained and dried at same time and held at room temp. 7 days for tests on germicide to be investigated for sporicidal activity. (Spores dried and held under these conditions will retain their resistance for 7 days or longer.)

Place 10 ml disinfectant at diln recommended for use or under investigation in 25×150 mm lipped test tube. Place tube in 20° H_2O bath and let come to temp. Select set of 5 dried, contaminated suture loops or cylinders shown to be carrying resistant spores of the culture for use in tests on each diln of disinfectant. Transfer 1 suture loop or cylinder immediately to tube of thioglycollate subculture medium as viability control. Then transfer remaining 4 loops to the diln of disinfectant in H_2O bath. Remove individual loops or cylinders at 4 selected time intervals, for example,

10, 30, 60, and 120 min., transferring them to individual tubes of thioglycollate subculture medium or other subculture medium specified in **5.001**(a)(3), whichever may contain the most suitable neutralizer. Shake all tubes thoroly and incubate 1 week at 37°. If no growth occurs, and if there is reason to suspect that lack of growth may be due to bacteriostasis, transfer each loop or cylinder to fresh tube of medium and incubate at 37° for second period of 7 days. Report results as + (growth) or − (no growth) values.

Dilns of unknown germicides found to be effective against specific spores in this test using suture loop carrier may be expected to be effective in disinfecting against same spores in general premise applications; dilns found effective using porcelain cylinder carriers may be expected to be effective in disinfecting against same spores, carried by hard non-porous surfaces, providing contact periods are adequate. Killing in 10 out of 10 trials at the diln and time specified is considered as satisfactory evidence of desired response altho for confidence level of 95%, killing in 50 out of 50 replicates is required.

Fungicidal Test (*5*)—Official

(Applicable for use with water-miscible type fungicides used to disinfect inanimate objects.)

Using Trichophyton interdigitale

5.016 TEST ORGANISM

Use as test fungus typical strain of *Trichophyton interdigitale* isolated from dermatophytosis of foot. (Strain must sporulate freely on artificial media, presence of abundant conidia being manifested by powdery appearance on surface of 10-day culture, particularly at top of agar slant, and confirmed by microscopic examination. Conidia-bearing mycelium should peel easily from surface of dextrose agar. Conidia of required resistance survive 10 min. exposure at 20° to phenol diln of 1:60, but not to one of 1:45. Strain No. 640, ATCC, is suitable.)

5.017 CULTURE MEDIUM

Carry fungus on agar slants of following composition: Dextrose 2%, Neopeptone (Difco) 1%, agar 2%, adjusted to pH 6.1–6.3. Use same culture medium to prep. cultures for obtaining conidial suspension, and use fluid medium of same nutrient composition (without agar) to test viability of conidia after exposure to fungicide.

5.018 CARE OF FUNGUS STRAIN

Store stock culture of fungus on dextrose agar slants at 2–5°. At intervals not >3 months, transfer it to fresh agar slants, incubate 10 days at 25–30°, and then store at 2–5° until next transfer period. Do not use culture that has been kept at or above room temp. >10 days as source of inoculum for culture. (Cultures may be kept at room temp. to preserve strain and to inoculate cultures if transferred at intervals not >10 days.)

5.019 PREPARATION OF CONIDIAL SUSPENSION

Prep. petri dish cultures by planting inoculum at center of agar plate and incubating culture at 25–30° for 10, but not >15 days. Remove mycelial mats from surface of 5 agar plate cultures, using sterile spatula or heavy flattened wire. Transfer to heat-sterilized glass tissue grinder (A. H. Thomas Co. size B) and macerate with 25 ml sterile physiological NaCl soln (0.85% NaCl); or to heat-sterilized erlenmeyer contg 25 ml sterile saline with glass beads, and shake thoroly. Filter suspension thru sterile absorbent cotton to remove hyphal elements. Estimate density of conidial suspension by counting in hemacytometer and store at 2–10° as stock spore suspension (125–155 million conidia/ml) for periods up to 4 weeks for use in prepg test suspensions of conidia. Stdze test conidial suspensions as needed by dilg stock spore suspension with physiological NaCl soln so that it contains 5 million conidia/ml.

5.020 OPERATING TECHNIC

Prep. dilns of the fungicide. (Tests are similar to those described in **5.003**.) Place 5 ml of each fungicide soln and of phenol control solns in 25×150 mm test-culture tubes, arrange in order of ascending dilns, place tubes in 20° H_2O bath, and let come to temp. With graduated pipet place 0.5 ml spore suspension in first tube of fungicidal soln, shake, and immediately replace in H_2O bath; 30 sec. later add 0.5 ml conidial suspension to second tube. Repeat procedure at 30 sec. intervals for each fungicidal diln. If more convenient, run test at 20 sec. intervals. After 5, 10, and 15 min. exposure to fungicide, remove sample from each conidia-fungicide mixt. with 4 mm loop and place in 10 ml dextrose broth, **5.017**. To eliminate risk of faulty results due to possible fungistatic action, make subtransfers from the initial dextrose broth subculture tubes to fresh tubes of dextrose broth, using the 4 mm loop before incubation, or make initial subcultures in dextrose broth contg either 0.05% Na thioglycollate, 1.5% *iso*-octylphenoxy-polyethoxyethanol, or mixt. of 0.07% lecithin (Azolectin) and 0.5% sorbitan monoöleate ("Tween 80"), whichever gives lowest result. Incubate inoculated tubes at 25–30°. Read final results after 10 days, altho indicative reading can be made in 4 days.

NOTE: Highest diln that kills spores within 10 min. is commonly considered as highest diln that could be expected to disinfect inanimate surfaces contaminated with pathogenic fungi.

Germicidal and Detergent Sanitizers (6)—Official

(Suitable for detg the min. concn of germicide which can be permitted for use in sanitizing hard, non-porous surfaces. Twice this concn is min. recommended starting concn. Method also dets max. water hardness tolerances for recommended concns.)

5.021 REAGENTS

(**a**) *Culture media.*—(1) *Nutrient agar A.*—Boil 3 g beef ext., 5 g peptone (Bacto or equiv.; special grades must not be used), and 15 g agar in 1 L H_2O. Sterilize at 15 lb pressure 20 min. Use for daily transfer of test culture. (2) *Nutrient agar B.*—Prep. as above but use 30 g agar. Use for growing test cultures in French square bottles. (3) *Nutrient agar (AOAC).*—*See* **5.001(a)**(2). Use for prepg stock culture slants.

(**b**) *Subculture media.*—(1) Use tryptone glucose ext. agar (Difco), adding 25 ml stock neutralizer (**c**)/L. (2) Tryptone glucose ext. agar (Difco).

(**c**) *Neutralizer stock soln.*—Mix 40 g Azolectin, 280 ml Tween 80, and 1.25 ml phosphate buffer, (**e**); dil. with H_2O to 1 L and adjust to pH 7.2. Dispense in 100 ml quantities and sterilize 20 min. at 15 lbs.

(**d**) *Neutralizer blanks.*—For use with 200 ppm quaternary NH_4 compound or less. Mix 100 ml neutralizer stock soln, (**c**), 25 ml 0.25*M* phosphate buffer stock soln, (**e**), and 1675 ml H_2O. Dispense 9 ml portions into 20×150 mm test tubes. Sterilize 20 min. at 15 lbs.

(**e**) *Phosphate buffer stock soln.*—0.25*M*. Dissolve 34.0 g KH_2PO_4 in 500 ml H_2O, adjust to pH 7.2 with 1*N* NaOH, and dil. to 1 L.

(**f**) *Phosphate buffer dilution water.*—Add 1.25 ml 0.25*M* phosphate buffer stock soln, (**e**), to 1 L H_2O and dispense in 99 ml portions. Sterilize 20 min. at 15 lb pressure.

(**g**) *Test organisms.*—Use *Escherichia coli* ATCC No. 11229 or *Staphylococcus aureus* ATCC 6538. Maintain stock cultures on nutrient agar AOAC, (**a**)(3), at refrigerator temp.

5.022 RESISTANCE TO PHENOL OF TEST CULTURES

Det. resistance to phenol at least every 3 months by **5.001–5.005**. Resistance of *E. coli* should be equiv. to that specified for *S. typhosa* in **5.004** and that for *Staph. aureus* equiv. to that specified for this organism in **5.005**.

5.023 APPARATUS

(**a**) *Glassware.*—250 ml wide-mouth erlenmeyers; 100 ml graduate; Mohr pipets; 20×150 mm test tubes. Wash in strong, fresh cleaning soln, and fill and drain with H_2O at least 3 times. Sterilize at 180° in hot air oven at least 2 hr.

(**b**) *Petri dishes.*—Sterile.

(**c**) *French square bottles.*—175 ml, borosilicate.

(**d**) *Water bath.*—Thermostated or controlled at 25°.

5.024 PREPARATION OF CULTURE SUSPENSION

From the stock culture inoculate tube of nutrient agar *A*, (**a**)(1), and make at least 3 consecutive daily transfers (not more than 30), incubating transfers 20–24 hr at 35°. Do not use transfers for more than 30 days. If only 1 daily transfer has been missed, no special procedures are required; if 2 daily transfers are missed, repeat with 3 daily transfers.

Prep. 175 ml French square culture bottles contg 20 ml nutrient agar *B*, (**a**)(2), sterilize 20 min. at 15 lb, and let solidify with bottle in horizontal position. Inoculate culture bottles by washing growth from slant into 99 ml phosphate buffer diln water, (**f**), and adding 2 ml of this suspension to each culture bottle, tilting back and forth to distribute suspension; then drain off excess liquid. Incubate 18–24 hr at 35°, agar side down. Remove culture from agar surface of 4 or more bottles, using 3 ml phosphate buffer diln water and glass beads in each bottle to suspend the growth. Filter suspension thru Whatman No. 2 paper and collect in sterile tube. (Filtration may be hastened by rubbing paper gently with sterile policeman.) Stdze suspension to give av. of 10×10^9 organisms /ml by diln with sterile phosphate buffer diln water, (**f**).

If Lumetron colorimeter is used, dil. suspension in sterile Lumetron tube to give % light transmission according to table:

% LIGHT TRANSMISSION FILTERS, Mμ							AV. BACTERIAL COUNT/ML
370	420	490	530	550	580	650	
7.0	4.0	6.0	6.0	6.0	7.0	8.0	13.0×10^9
8.0	5.0	7.0	7.0	7.0	8.0	9.0	11.5
9.0	6.0	8.0	8.0	8.0	9.0	10.0	10.2
10.0	7.0	9.0	9.0	9.0	11.0	11.0	8.6
11.0	8.0	10.0	10.0	10.0	12.0	13.0	7.7
13.0	9.0	12.0	12.0	12.0	13.0	15.0	6.7

If McFarland nephelometer and $BaSO_4$ stds are used, select 7 tubes of same i.d. as that contg test culture suspension. Place 10 ml of each suspension of $BaSO_4$, prepd as indicated in table, in each tube and seal tube. Stdze suspension to correspond to No. 4 std:

STD NO.	ML 2% $BaCl_2$ SOLN	ML 1% H_2SO_4 (V/V) SOLN	AV. BACTERIAL COUNT, ML
1	4.0	96.0	5.0×10^9
2	5.0	95.0	7.5
3	6.0	94.0	8.5
4	7.0	93.0	10.0
5	8.0	92.0	12.0
6	10.0	90.0	13.5
7	12.0	88.0	15.0

5.025 SYNTHETIC HARD WATER

Prep. *Soln A* by dissolving 31.74 g $MgCl_2$ (or equiv. of hydrates) and 73.99 g $CaCl_2$ in boiled distd H_2O and dilg to 1 L. Prep. *Soln B* by dissolving 56.03 g $NaHCO_3$ in boiled distd H_2O and dilg to 1 L. Soln A may be heat sterilized; Soln B must be sterilized by filtration. Place required amount of Soln A in sterile 1 L flask and add at least 600 ml sterile distd H_2O; then add 4 ml Soln B and dil. to 1 L with sterile distd H_2O. Each ml Soln A will give a water equiv. to approx. 100 ppm of hardness calcd as $CaCO_3$ by formula:

Total hardness as ppm $CaCO_3 = 2.495\times$ppm Ca$+4.115\times$ppm Mg.

The pH of all test waters up to 2000 ppm hardness should be 7.6–8.0. Check prepd synthetic waters chemically for hardness at time tests are made, using following method or other methods described in 10th Ed. of *Standard Methods for the Examination of Water, Sewage, and Industrial Wastes*.

5.026 HARDNESS METHOD

(a) *EDTA std soln.*—Dissolve 4.0 g disodium dihydrogen ethylenediamine tetraacetate.$2H_2O$ and 0.10 g $MgCl_2.6H_2O$ in 800 ml H_2O and adjust by subsequent diln so that 1 ml of soln is equiv. to 1 mg $CaCO_3$ when titrd as in (**b**). Prep. std Ca soln (1 ml = 1 mg $CaCO_3$) by weighing 1 g $CaCO_3$, dried overnight or longer at 105°, into 500 ml erlenmeyer and adding thru funnel dil. HCl until $CaCO_3$ is dissolved. Add 200 ml H_2O, boil to expel CO_2, and cool. Add few drops Me red indicator and adjust to intermediate orange color with dil. NH_4OH or HCl as required. Transfer quantitatively to 1 L vol. flask and dil. to vol.

(b) *Determination.*—Dil. 5–25 ml sample (depending on hardness) to 50 ml with H_2O in erlenmeyer or casserole. Add 1 ml *buffer soln* (67.5 g NH_4Cl and 570 ml NH_4OH dild to 1 L with H_2O), 1 ml *inhibitor* (5.0 g $Na_2S.9H_2O$ or 3.7 g $Na_2S.5H_2O$ dissolved in 100 ml H_2O), and 1 or 2 drops *indicator soln* (0.5 g Chrome Black T in 100 ml 60–80% alcohol). Titrate with EDTA std soln slowly, stirring continuously, until last reddish tinge disappears from soln, adding last few drops at 3–5 sec. intervals. Hardness as mg $CaCO_3$/L = (ml std soln $\times$1000)/ml sample.

5.027 UNKNOWN SAMPLES

Use composition declared or detd as guide to sample wt required for vol. sterile H_2O used to prep. 20,000 ppm soln. From this stock diln, transfer 1 ml into 99 ml of the water to be used in test to give concn of 200 ppm. In making this transfer, fill 1 ml pipet and drain back into stock soln; then refill to correct for adsorption on glass. After mixing, discard 1 ml to provide 99 ml of the test water in **5.028**.

5.028 OPERATING TECHNIC

Measure 99 ml of the water to be used in test, contg bactericide at concn to be tested, into chemically clean, sterile, 250 ml wide-mouth erlenmeyer and place in constant temp. bath until it reaches 25°, or at least 20 min. Prep. duplicate flasks for each germicide to be tested. Also prep. similar flask contg 99 ml sterile phosphate buffer diln H_2O, (**f**), as "initial numbers" control.

Add 1 ml culture suspension to each test flask as follows: Whirl flask, stopping just before suspension is added, creating enough residual motion of liquid to prevent pooling of suspension at point of contact with test water. Add suspension midway between center and edge of surface with tip of pipet slightly immersed in test soln. Avoid touching pipet to neck or side of flask during addn. Transfer 1 ml portions of this exposed culture to neutralizer blanks exactly 30 and 60 sec. after addn of suspension. Mix well immediately after transfer.

For "numbers control" transfer, add 1 ml culture suspension to 99 ml sterile phosphate diln water in same manner. In case of numbers control, plants need be made only immediately after adding and thoro mixing not longer than 30 sec. (In performance of test, it is advantageous to use milk pipets for adding culture and withdrawing samples.)

Plate from neutralizer tube to agar, using subculture media (**b**)(1) for quaternary NH_4 compounds and (**b**)(2) with numbers control. Where 0.1 ml portions are planted, use 1 ml pipet graduated in 0.1 ml intervals. For dilns to give countable plates, use phosphate buffer diln H_2O, (**f**). For numbers control, use following diln procedure: Transfer 1 ml exposed culture (1 ml culture suspension transferred to 99 ml phosphate buffer diln water in H_2O bath) to 99 ml phosphate buffer diln water, (**f**), (*diln A*). Shake thoroly and transfer 1 ml diln A to 99 ml phosphate buffer diln water, (**f**),

(*diln B*). Shake thoroly and transfer 1 ml diln B to 99 ml phosphate buffer (*diln C*). Shake thoroly and transfer from diln C four 1 ml and four 0.1 ml aliquots to individual sterile petri dishes.

For test samples, use following diln procedure: Transfer 1 ml exposed culture into 9 ml neutralizer, (**d**). Shake and transfer four 1 ml and four 0.1 ml aliquots to individual sterile petri dishes. For numbers control, use tryptone glucose ext. agar, (**b**)(2); for tests with quaternary NH_4 compounds, use tryptone glucose ext. agar with neutralizer, (**b**)(1). Cool agar to solidify, and then invert and incubate 48 hr at 35° before counting.

5.029 RESULTS

Results to be considered valid must meet std effectiveness: 99.999% reduction in count of no. of organisms within 30 sec. Report results according to actual count and % reduction over numbers control. Counts on numbers control for germicide test mixt. should fall between 75 and 125 million/ml for % reductions to be considered valid.

5.030 STERILITY CONTROLS

(**a**) *Neutralizer*.—Plant 1 ml from previously unopened tube.

(**b**) *Water*.—Plate 1 ml from each type of water used.

(**c**) *Sterile distilled water*.—Plate 1 ml.

After counting plates, confirm that surviving organisms are *E. coli* by transfer to brilliant green bile broth fermentation tubes or lactose broth and EMB agar; confirm *Staph. aureus* by microscopic examination.

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(*2*) J. Bact. **49,** 526(1945); Am. J. Vet. Research **9,** 104(1948); J. Assoc. Offic. Agr. Chemists **36,** 466(1953).

(*3*) Soap Sanit. Chemicals **27,** No. 2, 133(1951); J. Assoc. Offic. Agr. Chemists **38,** 274(1955); ibid. **40,** 755(1957).

(*4*) J. Assoc. Offic. Agr. Chemists **36,** 480 (1953); **39,** 480(1956); **40,** 759(1957).

(*5*) Arch. Dermatol. and Syphilol. **28,** 15(1933); J. Bact. **42,** 225(1941); ibid. **47,** 102(1944); J. Assoc. Offic. Agr. Chemists **37,** 616(1954); ibid. **38,** 274(1955).

(*6*) Am. J. Public Health **38,** 1405(1948); J. Milk and Food Technol. **19,** 183(1956); Federal Register **21,** 7020(1956); J. Assoc. Offic. Agr. Chemists **41,** 541(1958).

6. Plants

6.001 Sampling (*1*)—Official

When more than one plant is sampled, include sufficient number of plants in sample to insure that it adequately represents av. composition of entire lot of plants sampled. (This number cannot be stated definitely; it depends upon variability in composition of the plants.) Det. details of sampling by purpose for which sample is taken.

6.002 Preparation of Sample (*1*)—Official

(**a**) *For mineral constituents.*—Thoroly remove all foreign matter from material, especially adhering soil or sand, but to prevent leaching, avoid excessive washing. Air- or oven-dry as rapidly as possible to prevent decomposition or loss in wt by respiration, grind, and preserve in tightly stoppered bottles. If results are to be expressed on fresh wt basis, record wts of sample before and after drying. When Cu, Mn, Zn, Fe, Al, etc. are to be detd, avoid contamination of sample by dust during drying and from grinding and sieving machinery.

(**b**) *For carbohydrates.*—Thoroly remove all foreign matter and rapidly grind or chop material into fine pieces. Add weighed sample to enough hot redistd alcohol to which enough pptd $CaCO_3$ has been added to neutralize acidity, using enough alcohol so that final concn, allowing for H_2O content of sample, is ca 80%. Heat nearly to b.p. on steam or H_2O bath 30 min., stirring frequently. (Samples may be stored until needed for analysis.)

6.003 Moisture—First Action—*See* 22.003, 22.007, or 22.008

6.004 Ash—First Action—*See* 29.012–29.013; 22.010

6.005 Sand and Silica—Official

Ignite 10–50 g sample in flat-bottom Pt dish in muffle, at 500–550°, until residue is white or nearly so. (Pt dishes must be used with caution in ashing plant materials high in Fe; for such materials, use well-glazed porcelain crucibles and run blank detn.) Moisten with 5–10 ml HCl, boil ca 2 min., evap. to dryness, and heat on steam bath 3 hr to render SiO_2 insol. Moisten residue with 5 ml HCl, boil 2 min., add ca 50 ml H_2O, heat on H_2O bath few min., filter thru hardened paper, and wash thoroly. To this filtrate add filtrate and washings from alkali-sol. SiO_2 detn (**b**) and dil. to 200 ml. Designate as Soln A.

(**a**) *Sand.*—Wash residue from filter into Pt dish and boil ca 5 min. with ca 20 ml satd Na_2CO_3 soln; add few drops 10% NaOH soln, let mixt. settle, and decant thru ignited and weighed gooch. Boil residue in dish with another 20 ml portion of the Na_2CO_3 soln and decant as before. Repeat process. Transfer residue to gooch and wash thoroly, first with hot H_2O, then with little HCl (1+4), and finally with hot H_2O until Cl-free. Dry filter and contents, ignite at 500–550°, and weigh as sand. Confirm by microscopic examination.

(**b**) *Alkali-soluble SiO_2.*—Combine alk. filtrate and washings, acidify with HCl, evap. to dryness, add 5 ml HCl, again evap., and dehydrate by heating 2 hr at 110–120°. Moisten residue with 5–10 ml HCl, boil ca 2 min., add ca 50 ml H_2O, heat on H_2O bath 10–15 min., filter thru ashless filter or ignited and weighed gooch, wash with hot H_2O, ignite at 500–550°, and weigh as SiO_2. Add filtrate to Soln A.

METALS

6.006 Iron and Aluminum (*2*)—Official

Take aliquot of Soln A, **6.005**, contg enough Fe and Al to form ca 40 mg Fe- and $AlPO_4$. Add few drops HNO_3, Br-H_2O, or H_2O_2 to oxidize Fe. If soln does not already contain excess phosphate, add 0.5 g $(NH_4)_2HPO_4$, stir until dissolved, and dil. to 50 ml with H_2O. Add few drops thymol blue soln, **35.095(k)**, and then add NH_4OH until soln just turns yellow. Add 0.5 ml HCl and 25 ml 25% NH_4OAc soln, and stir. Let stand at room temp. until ppt settles (ca 1 hr). Filter, and wash 10 times with hot 5% NH_4NO_3 soln. Ignite at 500–550° and weigh as $FePO_4$ and $AlPO_4$.

Fuse ignited ppt in Pt crucible with ca 4 g mixt. of equal parts Na_2CO_3 and K_2CO_3. When fusion is complete, let crucible cool, add 5 ml H_2SO_4, and heat until copious fumes of SO_3 are evolved. Cool, transfer to flask, add H_2O, and digest until soln is clear. Reduce Fe with Zn, cool, and titr. with 0.1*N* $KMnO_4$. Correct for blank and calc. as % Fe or % Fe_2O_3. Calc. to $FePO_4$ and subtract from total Fe- and $AlPO_4$ to obtain $AlPO_4$. Correct for blank and report as Al_2O_3.

Methods for Iron Only

Colorimetric Method (3)—Official

6.007 REAGENTS

(a) *Acetic acid.*—2*M*. Dil. 120 g HOAc to 1 L with H_2O.

(b) *Ammonium citrate soln.*—1%. Dissolve 1 g NH_4 citrate in H_2O and dil. to 100 ml.

(c) *Bromophenol blue indicator.*—0.04%. Grind 0.1 g bromophenol blue in mortar with 3 ml 0.05*N* NaOH, transfer to vol. flask, and dil. to 250 ml with H_2O.

(d) *Buffer solns*:

(1) pH 3.5.—Mix 6.4 ml 2*M* NaOAc with 93.6 ml of the 2*M* HOAc and dil. to 1 L.

(2) pH 4.5.—Mix 43 ml 2*M* NaOAc with 57 ml of the 2*M* HOAc and dil. to 1 L.

(e) *Hydroquinone soln.*—Dissolve 1 g hydroquinone in 100 ml pH 4.5 buffer, (d)(2). Keep in refrigerator or other cool place, and discard when any color develops.

(f) *o-Phenanthroline soln.*—Dissolve 1 g *o*-phenanthroline monohydrate in H_2O, warming if necessary, and dil. to 400 ml.

(g) *Sodium acetate soln.*—2*M*. Dissolve 272 g $NaOAc.3H_2O$ in H_2O and dil. to 1 L.

(h) *Iron std soln.*—1 mg Fe/ml. Dissolve 1 g electrolytic Fe in 50 ml 10% H_2SO_4, warming if necessary to hasten reaction. Cool, and dil. to 1 L with H_2O.

6.008 PREPARATION OF SAMPLE

Use Soln A, or if Soln A is not available, weigh samples of finely ground plant material (1–5 g, depending on Fe content) into porcelain crucibles with smooth inner surfaces, and ash overnight at 500–550° in muffle. Cool, add 5 ml HCl (1+1), and heat on steam bath 15 min. to dissolve Fe and to hydrolyze pyrophosphate. Filter into 100 ml vol. flask. Transfer insol. residue to filter and wash 5 times with 3 ml portions hot HCl (1+100), then with hot H_2O until washings are Cl-free. Ignite paper and any remaining C in Fe-free Pt crucible. Cool, add 2 drops H_2SO_4 and 1 ml HF, and carefully evap. to SO_3 fumes. Cool, add few drops HCl (1+1), and warm. Filter and wash as before into same vol. flask, dil. to vol., and mix.

6.009 DETERMINATION

Pipet identical aliquots of Soln A, **6.005**, or sample soln, **6.008**, into 25 ml vol. flask and into test tube or small erlenmeyer. Add 5 drops of the bromophenol blue indicator to aliquot in test tube, and titr. with the NaOAc soln until color matches that of equal vol. of pH 3.5 buffer contg same quantity of indicator. Add 1 ml of the hydroquinone soln and 2 ml of the *o*-phenanthroline soln to aliquot in vol. flask, and adjust pH of contents to 3.5 by adding same vol. NaOAc soln found necessary for aliquot in test tube. If turbidity develops upon adjusting pH of aliquot in test tube, add 1 ml of the NH_4 citrate soln to vol. flask before adding the NaOAc soln. Dil. to vol., mix, and let stand 1 hr to assure complete color development. Det. Fe colorimetrically.

Select aliquot contg quantity of Fe suitable for range of colorimeter to be used.

For photoelec. colorimeter, this quantity depends on light filter (470–520 mμ) and thickness of absorption cells used. No. 430 dark-shade, blue-green, Corning glass light filter, ca 12.5 mm thick (obtained by using 2 molded filters of half this thickness) is satisfactory, and when used with 1 cm absorption cells in Cenco-Sheard-Sanford photelometer, reliable range is 0.02–0.1 mg Fe /25 ml soln.

For spectrophotometer, prep. curve relating transmittance to mg Fe in 25 ml by treating series of solns contg varying quantities of Fe that cover usable range of instrument exactly as described for unknowns, detg their respective transmittance readings, and plotting these against corresponding concns of Fe. H_2O may be used as reference, and blanks detd to correct for quantity of Fe in reagents used, or blank soln itself may be made basis of comparison.

For visual colorimeter, range of 0.2–0.5 mg Fe/25 ml is suggested. Prep. series of stds covering this range simultaneously with unknowns and compare each unknown with std that does not vary >25% from it in concn.

6.010 *Titrimetric Method (4)—Official*

Take appropriate aliquot of Soln A or of soln prepd as in **6.008**, and oxidize the Fe by adding dropwise soln of $KMnO_4$ (1+1000) until very faint permanganate color persists. Add 5 ml 10% NH_4CNS and titr. with *dil. $TiCl_3$ soln* until red color disappears. (To prep. appropriate $TiCl_3$ soln, boil 5–10 ml 20% $TiCl_3$ with 50 ml HCl few min., cool, and dil. to 1 L. Stdze this soln against std Fe soln, keep in dark in well-filled container, and stdze against the Fe soln each time it is used, or every few hr when many detns are being made. Discard when decomposition is indicated by loss of color and increased titer against std.)

Calcium—Official

6.011 *Macro Method (5)*

Transfer aliquot of Soln A, **6.005**, to 200 ml beaker, add H_2O if necessary to make to 50 ml, heat to boiling, and add 10 ml satd $(NH_4)_2C_2O_4$ soln and drop Me red, **2.034**(i). Almost neutralize with NH_4OH and boil until ppt is coarsely granular. Cool, add NH_4OH (1+4) until color is faint pink (pH 5.0), and let stand at least 4 hr.

Filter, and wash with H_2O at room temp. until filtrate is oxalate-free. Break point of filter with Pt wire, and wash ppt into beaker in which the Ca was pptd, using stream of hot H_2O. Add ca 10 ml H_2SO_4 (1+4), heat to ca 90°, add ca 50 ml hot H_2O, and titr. with 0.05*N* $KMnO_4$. Finally add filter paper to soln and complete titrn.

6.012 *Micro Method* (*6*)

Weigh 2 g sample into small crucible and ignite in muffle at 500–550°. Dissolve ash in HCl (1+4) and transfer to 100 ml beaker. Add 5 ml HCl and evap. to dryness on steam bath to dehydrate SiO_2. Moisten residue with 5 ml HCl, add ca 50 ml H_2O, heat few min. on steam bath, transfer to 100 ml vol. flask, cool quickly to room temp., dil. to vol., shake, and filter, discarding first portion of filtrate.

Pipet 15 ml aliquot into conical-tip centrifuge tube contg 2 ml satd $(NH_4)_2C_2O_4$ soln and 2 drops Me red, **2.034**(i). Add 2 ml HOAc (1+4), rotating tube to mix contents thoroly. Add NH_4OH (1+4), while intermittently rotating tube, until soln is faintly alk.; then add few drops of the HOAc from dropper until color is faint pink (pH 5.0). (It is important at this point to rotate tube so that last bit of liquid in conical tip has required color.) Let stand at least 4 hr; then centrifuge 15 min. (Ppt should be in firm lump in tip of tube.) Remove supernatant, using suction device, Fig. 14, taking care not to disturb ppt. Wash ppt by adding 2 ml NH_4OH (1+49), rotating tube to break up ppt. (It may be necessary to jar tube sharply.) Centrifuge 10 min., again remove supernatant, and wash with reagent as before. Repeat this operation of washing ppt 3 times.

After last supernatant has been removed, add 2 ml H_2SO_4 (1+4) to tube, break up ppt as before, heat on steam bath to 80–90°, and titr. in tube with 0.02*N* $KMnO_4$, rotating liquid during titrn to attain proper end point. If tube cools to <60° during titrn, as indicated by slow reduction of $KMnO_4$, reheat in steam bath few min. and complete titrn. Perform blank on identical quantity H_2SO_4 in similar tube heated to same temp. to det. quantity of the $KMnO_4$ soln necessary to give color of end point. Subtract this value from buret reading. 1 ml 0.02*N* $KMnO_4$ = 0.000400 g Ca. Report as % Ca.

6.013 **Magnesium** (*7*)—**Official**

To combined filtrate and washings from Ca detn, **6.011**, add 30 ml HNO_3 and evap. to dryness to decompose NH_4 salts. Take up with 5 ml HCl and dil. to ca 100 ml with H_2O. Add 5 ml *10% Na citrate soln* and 10 ml *10%* $(NH_4)_2HPO_4$ *soln*, or enough to ppt all the Mg. Add NH_4OH (1+4) with constant stirring (using policeman) until soln is faintly alk. and ppt forms; then add 25 ml

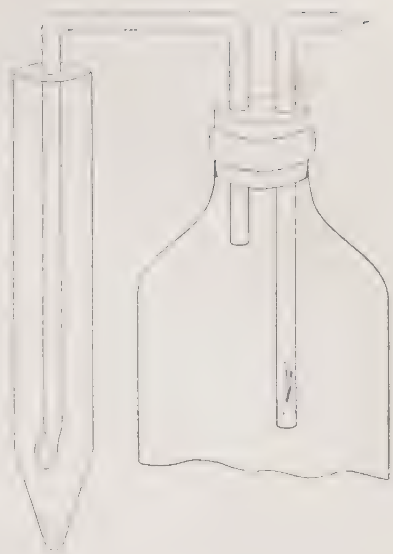

FIG. 14.—SUCTION DEVICE USED IN MICRO METHOD FOR DETERMINATION OF CALCIUM

NH_4OH, stir vigorously until ppt is granular, and keep in cool place overnight. Filter, and wash free from Cl with cold NH_4OH (1+10). Ignite in muffle at 500–550° until all C is oxidized, then at 900–950° for approx. 4 hr to form $Mg_2P_2O_7$. Cool, and weigh as $Mg_2P_2O_7$. (If sample is excessively high in Mn, dissolve ignited ppt in HNO_3, det. Mn as in **6.014**, and correct $Mg_2P_2O_7$ for $Mn_2P_2O_7$.) Report as % Mg.

6.014 **Manganese** (*8*)—**Official**

To aliquot of Soln A, **6.005**, equiv. to 0.2–0.5 g ash, add 15 ml H_2SO_4 and evap. to ca 30 ml. Add 5–10 ml HNO_3 and continue evapn. (Do not evap. until dense fumes appear, because $Fe_2(SO_4)_3$ then dissolves with difficulty. HNO_3 may be present, but not HCl.) Add H_2O, little at time, heat until Fe salts dissolve, and dil. to ca 150 ml. Add 0.3 g KIO_4, or its equiv. in HIO_4, in small portions, boil few min. or until color of $KMnO_4$ shows no further increase in intensity, and let cool.

Prep. std as follows: To vol. H_2O equal to sample add 15 ml H_2SO_4 and enough pure $Fe(NO_3)_3$, free from Mn, to equal ca quantity of Fe in sample. Add measured quantity of 0.1*N* $KMnO_4$ until color is slightly darker than sample, then add 0.3 g KIO_4, and boil few min. When cool, transfer sample and std to 250 ml flasks and dil. to mark with H_2O. (If color is weak, it may be necessary to dil. to <250 ml.) Compare colors in colorimeter. (Photoelec. colorimeter with 525–550 mμ light filter may be used.) Report results as % Mn.

6.015 **Sodium and Potassium—Official**

Moisten 1–10 g sample with H_2SO_4 (1+10), dry in oven, and ignite in muffle at 500–550° to

destroy org. matter. Heat residue on steam bath with 2–5 ml HCl and ca 50 ml H_2O. Transfer to beaker and add NH_4OH dropwise until ppt formed requires several sec. to dissolve, thus leaving soln only faintly acid. Heat nearly to boiling, and add NH_4OH to ppt all Fe, Al, etc. Boil in covered beaker ca 1 min.; remove, and if no NH_3 is detected by smelling, continue addn, dropwise, until it can be detected. Stir and without letting ppt settle, pour on filter. Wash immediately with hot H_2O, using, to effect rapid filtration, fine jet directed around edge of ppt to cut it free from the paper. Wash ppt several times, return to original beaker, dissolve with few drops HCl, and warm. Reppt Fe, Al, and P_2O_5 with NH_4OH as above; filter, and wash until free from Cl.

Evap. combined filtrates and washings to dryness, heat <450° until NH_4 salts are expelled, and dissolve in hot H_2O. Add 5 ml satd $Ba(OH)_2$ soln, heat to boiling, let settle few min., and det. if pptn is complete by adding more $Ba(OH)_2$ soln to little clear liquid. When no further ppt is produced, filter and wash thoroly with hot H_2O.

Heat filtrate to boiling and add NH_4OH (1+4) and 10% $(NH_4)_2CO_3$ soln to complete pptn of Ba, Ca, etc. Let stand short time on H_2O bath, filter, and wash ppt thoroly with hot H_2O. Evap. filtrate and washings to dryness, expel NH_4 salts by heating <450°, treat with little hot H_2O, and add few drops of the dil. NH_4OH, 1 or 2 drops of the $(NH_4)_2CO_3$ soln, and few drops satd NH_4 oxalate soln. Let stand few min. on H_2O bath and set aside few hr. Filter, evap. to complete dryness on H_2O bath, and heat at not >500° until all NH_4 salts are expelled and residue is nearly or quite white. Dissolve in min. quantity H_2O, filter into weighed Pt dish, add few drops HCl, evap. to dryness on H_2O bath, heat at not >500°, cool in desiccator, and weigh as KCl+NaCl. Repeat heating to constant wt.

Sodium and/or Potassium

Flame Photometric Method (9)—First Action

6.016 REAGENTS

(a) *Potassium stock soln.*—1000 ppm K. Dissolve 1.907 g dry KCl in H_2O and dil. to 1 L.

(b) *Sodium stock soln.*—1000 ppm Na. Dissolve 2.542 g dry NaCl in H_2O and dil. to 1 L.

(c) *Lithium stock soln.*—1000 ppm Li. Dissolve 6.110 g LiCl in H_2O and dil. to 1 L. (Needed only if internal std method of evaluation is to be used.)

(d) *Ammonium oxalate stock soln.*—0.24*N*. Dissolve 17.0 g $(NH_4)_2C_2O_4.H_2O$ in H_2O and dil. to 1 L.

(e) *Extracting solns.*—(1) *For potassium.*—For internal std method dil. required vol. of the LiCl stock soln to 1 L; otherwise use H_2O. (2) *For sodium.*—To 250 ml of the NH_4 oxalate stock soln add required vol. of the LiCl stock soln (if internal std method is used) and dil. to 1 L. If internal std requirements are same for both Na and K detns, this reagent may be used as common extg soln.

6.017 PREPARATION OF STANDARD SOLUTIONS

Dil. appropriate aliquots of the stock solns to prep. series of stds contg K and/or Na in stepped amounts (including 0) to cover instrument range, and Li and NH_4 oxalate (if required) in same concns as in corresponding extg solns. (If common extg soln is used, 1 set of stds contg both K and Na will suffice.)

6.018 SAMPLE EXTRACTION

Transfer weighed portion of finely ground and well-mixed sample to erlenmeyer of at least twice capacity of vol. of extg soln to be used. Add measured vol. extg soln, stopper flask, and shake vigorously at frequent intervals during at least 15 min. Filter thru dry, fast paper. If paper clogs, pour contents onto addnl fresh paper and combine filtrates. Use filtrate for detn.

NOTE: Do not make exts more concd than required for instrument, because there is tendency toward incomplete extn as ratio of sample wt to vol. extg soln increases. Prep. sep. exts for K and Na when their concns in sample differ greatly. For K use wt sample not >0.1 g/50 ml extg soln; for low Na concns use at least 1.0 g/50 ml extg soln; and for higher concns prep. weaker exts by reducing ratio of sample to extg soln rather than by dilg stronger exts.

6.019 DETERMINATION

Rinse all glassware used in Na detn with dil. HNO_3, followed by several portions H_2O. Protect solns from air-borne Na contamination.

Operate instrument in accordance with instructions of manufacturer. Permit instrument to reach operating equilibrium before use. Atomize portions of the std solns toward end of warm-up period until reproducible readings for series are obtained.

Run stds, covering concn range of samples involved, at frequent intervals during atomization of series of sample solns. Repeat this operation with both std and sample solns enough times to result in reliable av. reading for each soln. Plot analysis curves from readings of stds, and calc. % K and/or Na in samples.

Potassium

6.020 *Platinic Chloride Method—Official*

Dissolve residue of mixed chlorides, **6.015**, in few ml H_2O, acidify with few drops HCl, and add excess H_2PtCl_6 soln, **2.059(b)**. Evap. on H_2O bath

to thick paste; treat residue repeatedly with 80% alcohol, decanting thru weighed gooch or other form of filter; transfer ppt to filter; and wash with 80% alcohol until filtrate is colorless. Dry 30 min. at 100° and weigh. $K_2PtCl_6 \times 0.1609 = K$. If Na is desired, calc. K to KCl and subtract this from the KCl+NaCl, **6.015.**

6.021 *Perchloric Acid Method (10)—Official*

Prep. sample as in **6.015,** up to point where heavy metals have been removed and Na and K are in form of chlorides. (Sulfates must be absent.) Add 3–5 ml 60% $HClO_4$. Evap. to dryness, dissolve in hot H_2O, and again evap. to dryness. Heat to 350°, cool, and weigh, if combined perchlorates are desired. Add 10–20 ml anhyd. *EtOAc-n-butanol* (1+1). Digest near b.p. several min. Decant into gooch. Wash once or twice by decanting with few ml of the EtOAc-butanol mixt. Dissolve in min. quantity H_2O, evap. to dryness, and ext. as before. Filter, and wash several times with 1 ml of the EtOAc-butanol mixt. Dry in oven at 110° several min. and heat 15 min. at 350°. Cool and weigh. $KClO_4 \times 0.2822 = K$. Calc. Na by difference.

6.022 *Rapid Method for Potassium Only—Official*

Proceed as in **6.015** thru "and if no NH_3 is detected . . . until it can be detected." Add few ml satd $(NH_4)_2CO_3$ soln, let stand few hr, filter, and wash with hot H_2O until Cl-free. Conc. to small vol., transfer to Pt evapg dish, evap. to drive off excess NH_3, add 0.5 ml H_2SO_4 (1+1), evap., ignite by swirling dish over free flame, and proceed as in **6.020.**

Sodium Only

Uranyl Acetate Method (11)—Official

6.023 REAGENT

Magnesium uranyl acetate soln:

(**a**) *Uranyl acetate soln.*—To 85 g $UO_2(OAc)_2.2H_2O$ in 1 L vol. flask add 60 g HOAc and H_2O to ca 900 ml. Heat to dissolve, cool, and dil. to mark with H_2O.

(**b**) *Magnesium acetate soln.*—To 500 g $Mg(OAc)_2.4H_2O$ in 1 L vol. flask add 60 g HOAc and H_2O to ca 900 ml. Heat to dissolve, cool, and dil. to mark with H_2O.

Reheat (**a**) and (**b**) separately to ca 70° until all salts dissolve. Mix 2 solns at this temp. and let cool to ca 30°. Place vessel contg mixed reagent in H_2O at 20°, and hold at this temp. 1–2 hr, or until slight excess of salts has crystd out. Filter reagent thru dry filter into dry bottle.

6.024 DETERMINATION

Moisten 1–10 g sample with H_2SO_4 (1+10), dry in oven, and ignite in muffle at 500–550° to destroy org. matter. Heat residue on steam bath with 2–5 ml HCl, add ca 40 ml H_2O, and heat to boiling. Add enough 5% $CaCl_2$ soln to insure pptn of all phosphates. Ppt phosphates by making slightly alk. with NH_4OH. Filter, and evap. to 5 ml or less if no salts sep. Cool, add 100 ml of the Mg uranyl acetate soln, place mixt. in H_2O bath at 20°, and either stir vigorously 45 min. or let stand 24 hr at this temp. Filter with suction, and wash with *alcohol satd with Na-Mg-uranyl acetate.* Dry 30 min. at 105–110°, cool, and weigh. Wt Na-Mg-uranyl acetate $\times 0.0153$ = wt Na.

Cobalt

Nitrosocresol Method (12)—First Action

6.025 REAGENTS

(Make all distns in Pyrex stills with ₮ joints. Store reagents in g-s. Pyrex bottles.)

(**a**) *Redistilled water.*—Distill twice, or pass thru column of ion-exchange resin (IR-100A, H-form, or equiv.) to remove heavy metals.

(**b**) *Hydrofluoric acid.*—Reagent grade, 48%. Procurement in vinyl plastic bottles is advantageous.

(**c**) *Perchloric acid.*—Reagent grade, 60%. No further purification necessary.

(**d**) *Hydrochloric acid.*—1+1. Add equal vol. reagent grade concd HCl to distd H_2O and distill.

(**e**) *Ammonium hydroxide.*—1+1. Distill concd NH_4OH into equal vol. redistd H_2O.

(**f**) *Ammonium hydroxide.*—0.02*N*. Add 7 ml of the NH_4OH (1+1) to 2.5 L redistd H_2O.

(**g**) *Carbon tetrachloride.*—Distill over CaO, passing distillate thru dry, acid-washed filter paper. Used CCl_4 may be recovered as in **6.040**(**a**).

(**h**) *Dithizone.*—Dissolve 0.5 g dithizone in 600–700 ml CCl_4 (tech. grade is satisfactory). Filter into 5 L separator contg 2.5–3.0 L 0.02*N* NH_4OH, shake well, and discard CCl_4 layer. Shake with 50 ml portions redistd CCl_4 until CCl_4 phase as it seps has pure green color. Add 1 L redistd CCl_4 and acidify slightly with the HCl (1+1). Shake the dithizone into CCl_4 layer and discard aq. layer. Store in cool, dark place, preferably in refrigerator.

(**i**) *Ammonium citrate soln.*—40%. Dissolve 800 g citric acid in 600 ml distd H_2O, and, while stirring, add slowly 900 ml NH_4OH. Reaction is exothermic and care must be taken to prevent spattering. Adjust pH to 8.5 if necessary. Dil. to 2 L and ext. with 25 ml portions dithizone soln until aq. phase stays orange and CCl_4 remains predominantly green. Then ext. soln with CCl_4 until all orange color is removed.

(j) *Hydrochloric acid*.—0.1N. Dil. 16.6 ml of the HCl (1+1) to 1 L with redistd H_2O.

(k) *Hydrochloric acid*.—0.01N. Dil. 100 ml of the 0.1N HCl to 1 L with redistd H_2O.

(l) *Sodium hydroxide soln*.—1N. Dissolve 40 g NaOH in 1 L redistd H_2O.

(m) *Sodium borate buffer*.—pH 7.8. Dissolve 20 g H_3BO_3 in 1 L redistd H_2O. Add 50 ml 1N NaOH and adjust pH if necessary. Equal vols Na borate buffer and 0.01N HCl should give soln of pH 7.0.

(n) *Sodium borate buffer*.—pH 9.1. To 1 L Na borate buffer, pH 7.8, add 120 ml 1N NaOH and adjust pH if necessary.

(o) *Skellysolve B*.—Essentially n-hexane. Purify by adding 20–30 g silica gel/L, let stand several days, and distill. Available from Skelly Oil Co., Solvents Division, Kansas City, Mo.

(p) *Cupric acetate soln*.—Dissolve 10 g $Cu(OAc)_2.H_2O$ in 1 L redistd H_2O.

(q) *o-Nitrosocresol*.—Dissolve 8.4 g anhyd. $CuCl_2$ and 8.4 g $NH_2OH.HCl$ in 900 ml H_2O. Add 8 ml m-cresol (Eastman, practical grade) and stir vigorously while slowly adding 24 ml 30% H_2O_2. Stir with motor-driven stirrer 2 hr at room temp. (Standing for longer periods results in excessive decomposition.) Add 25 ml HCl and ext. the o-nitrosocresol with four 150 ml portions Skellysolve B, (o), in large separator. Then add addnl 25 ml HCl and again ext. with four 150 ml portions Skellysolve B. Wash combined Skellysolve B exts twice with 50–100 ml portions 0.1N HCl and twice with 50–100 ml portions redistd H_2O. Shake o-nitrosocresol soln with successive 50–100 ml portions 1% $Cu(OAc)_2$ soln until aq. phase is no longer deep blood-red. When light purple color is evident, extn is complete. Discard Skellysolve B phase, acidify aq. soln of Cu salt with 25 ml HCl, and ext. reagent with two 500 ml portions of the Skellysolve B; wash twice with 150–200 ml portions of the 0.1N HCl and several times with 150–200 ml portions redistd H_2O. Store o-nitrosocresol soln in refrigerator at ca 4°. This reagent is stable 6 months or more.

(r) *Sodium o-nitrosocresol*.—Ext. 100 ml of the o-nitrosocresol by shaking with two 50 ml portions Na borate buffer, pH 9.1, in separator. (If this is carried out as 2 extns, resulting reagent is more concd. It is important that total vol. o-nitrosocresol soln equals total vol. buffer.)

(s) *Cobalt std soln*.—Heat $CoSO_4.7H_2O$ in oven at 250–300° to constant wt (6–8 hr). Weigh exactly 0.263 g of the $CoSO_4$ and dissolve in 50 ml redistd H_2O and 1 ml H_2SO_4. Dil. to 1 L. Transfer 5 ml of the stock soln to 1 L vol. flask and dil. to vol. with redistd H_2O. (1 ml = 0.5 mmg Co.)

(t) *Hydroxylamine acetate buffer*.—pH 5.1. Dissolve 10 g $NH_2OH.HCl$ and 9.5 g anhyd. NaOAc in 500 ml redistd H_2O. Resulting soln has pH of 5.0–5.2.

6.026 APPARATUS

(a) *Platinum dishes*.—Approx. 70 ml; for ashing.

(b) *Automatic dispensing burets*.—100 ml; type that can be fitted to ordinary 5 lb reagent bottle and filled by means of aspirator bulb is most convenient.

(c) *Wooden separator rack*.—Twelve 120 ml separator size is convenient for dithizone extns. Rack is fitted across top with removable bar padded with sponge rubber so all 12 separators can be shaken as unit.

(d) *Racks*.—Consisting of 2″×2″×25″ wooden bars with holes drilled at close intervals to take 50 ml centrifuge tubes fitted with No. 13 ⚲ glass stoppers are convenient. To make these tubes, ream out necks of heavy-wall Pyrex centrifuge tubes (Rockefeller Institute type) with ⚲ C rod and grind to take ⚲ stopper. Place tubes upright in one section, and place other section (fitted with sponge rubber disks ½″ thick in bottom of holes) across their tops. Fasten 2 sections at ends with removable rubber connectors made from ordinary tubing of convenient size, so that any number of tubes can be shaken as unit. Use these tubes for reaction of Co with nitrosocresol, extn of complex into Skellysolve B, and washing of Skellysolve B soln.

(e) *Shaking machine*.—Commercial mechanical shaker similar to Model L-2549, Catalog G-3 of George H. Wahmann Manufacturing Co., 1123 E. Baltimore St., Baltimore, Md., is satisfactory when modified to give longitudinal stroke of 2″ at ca 180 strokes/min. Use this shaker to make dithizone extns and to ext. Co complex.

(f) *Colorimeter*.—Suitable for measuring transmittance at ca 345 mμ. Colorimeter described by Ellis and Brandt (*13*) and Coleman Model 11 spectrophotometer, using null-point method, have been found satisfactory. With latter instrument, calibration curve deviates slightly from linearity, but region between 0 and 1 mmg Co approaches straight line. Use matched pair of Pyrex absorption cells at least 5 cm long; American Instrument Co. Catalog No. 5-997, Style D, horizontal, with neck for cork or rubber stopper is satisfactory; o.d. 13 mm, length 5 cm, and capacity ca 3 ml.

6.027 CLEANING OF GLASSWARE

Clean 120 ml Pyrex separators for dithizone extns by initially soaking 30 min. in hot HNO_3 and rinsing several times with H_2O. As added precaution, shake with several portions dithizone in CCl_4. After use, clean by rinsing with H_2O, drain,

and stopper to avoid contamination. It is not necessary to clean every time with acid. Repeat HNO_3 cleaning if blanks are unusually high.

Clean the 50 ml g-s. Pyrex centrifuge tubes by soaking 30 min. in HNO_3 followed by several rinsings in H_2O.

Completely submerge pipets in cylinder of H_2SO_4-$K_2Cr_2O_7$ cleaning soln overnight, rinse several times with H_2O, and suspend upright in rack to dry.

Wash all other glassware thoroly in detergent and rinse well with tap H_2O followed by dip in H_2SO_4-$K_2Cr_2O_7$ cleaning soln. Rinse off cleaning soln with tap H_2O followed by several distd H_2O rinses.

Clean Pt by scrubbing with sea sand followed by boiling in HCl (1+2) 30 min., and rinse several times with H_2O.

6.028 PREPARATION OF SAMPLE

See **6.002(a)**. Oven-dry all plant material 48 hr and prep. for ashing by either of following methods:

(a) Grind material in Wiley mill equipped with stainless steel sieve, mix thoroly by rolling, and sample by quartering.

(b) Using stainless steel shears, cut material by hand fine enough for convenient sampling.

6.029 ASHING OF SAMPLES

Weigh 6 g dry plant tissue into clean Pt dish. Cover with Pyrex watch glass and place in cool muffle; heat slowly to 500° and hold at this temp. overnight. Remove sample and cool. Wet down ash carefully with fine stream redistd H_2O. From dispensing buret add slowly 2–5 ml $HClO_4$, dropwise at first to prevent spattering. Add ca 5 ml HF, evap. on steam bath, transfer to sand bath, and keep at medium heat until fuming ceases.

Cover with Pyrex watch glass, return to partially cooled muffle, heat gradually to 600°, and keep at this temp. 1 hr. Remove sample and cool. Add 5 ml HCl (1+1) and ca 10 ml redistd H_2O. Replace cover glass and warm on steam bath to dissolve. (Usually clear soln essentially free of insol. material is obtained.) Transfer sample to 50 ml vol. flask, washing dish several times with redistd H_2O, dil. to vol., and mix thoroly. (Pt dishes can ordinarily be used several times between sand and acid cleanings.)

6.030 DITHIZONE EXTRACTION

Transfer suitable aliquot (2–3 g dry material) to 120 ml separator (use Vaseline as stopcock lubricant). Add 5 ml of the NH_4 citrate soln and 1 drop phthln; adjust to pH 8.5 with the NH_4OH (1+1). If ppt forms, add addnl NH_4 citrate. Add 10 ml of the dithizone in CCl_4 and shake 5 min. Drain CCl_4 phase into 100 ml beaker. Repeat as many times as necessary, using 5 ml quantities of dithizone soln and shaking 5 min. each time. Extn is complete when aq. phase remains orange and CCl_4 phase remains predominantly green. Then add 10 ml CCl_4, shake 5 min., and combine with CCl_4 ext. Final 10 ml CCl_4 should be pure green. If not, extn was incomplete and must be repeated.

Add 2 ml $HClO_4$ to combined CCl_4 exts, cover beaker with Pyrex watch glass, and digest on hot plate until colorless. Remove cover glass and evap. slowly to dryness. (If sample is heated any length of time at high temp. when dry, losses of Co may occur. Heat only enough to evap. completely to dryness. If free acid remains, it interferes with next step where pH control is important.)

Add 5 ml 0.01*N* HCl to residue. Heat slightly to assure soln. If Cu is to be detd, transfer with redistd H_2O to 25 ml vol. flask, and dil. to vol. Transfer 20 ml aliquot to 50 ml g-s. centrifuge tube or 60 ml separator and reserve remainder for Cu detn, **6.039**. If Cu is not to be detd, transfer entire acid soln with redistd H_2O to centrifuge tube or separator.

6.031 DETERMINATION

Add 5 ml of the Na borate buffer, pH 7.8, and 2 ml of the freshly prepd Na *o*-nitrosocresol soln to sample soln. Add exactly 5 ml Skellysolve B and shake 10 min. Remove aq. phase by moderate suction thru finely-drawn glass tube. To Skellysolve B layer add 5 ml of the $Cu(OAc)_2$ soln and shake 1 min. to remove excess reagent. Again remove and discard aq. phase. Wash Skellysolve B by shaking 1 min., with 5 ml redistd H_2O, removing aq. layer as before; finally shake Skellysolve B 1 min. with 5 ml of the hydroxylamine-NaOAc buffer to reduce Fe. Transfer Skellysolve B soln of the Co complex to 5 cm absorption cell and read in photoelec. colorimeter, using Corning std thickness filters Nos. 5860 and 4308, or light band as close as possible to point of max. absorption, 360 mμ.

6.032 BLANKS AND STANDARDS

With each set of detns include ashing blank, reagent blank, and appropriate std curve. Since Beer's law holds for Co-*o*-nitrosocresol complex, 3 points, 0.0 (reagent blank), 0.5, and 1.0 mmg, are enough to define std curve. Transmittance of 0.0 mmg point should never drop $<90\%$. If below, repurify *o*-nitrosocresol by transferring alternately to aq. phase as Cu salt and to Skellysolve B phase as free compound after acidifying aq. phase. Ashing blank should have transmittance not >2–3% lower than reagent blank.

It is also advisable to include std sample with each set of samples to detect contamination or

unusual losses of Co in method. Commercial buckwheat flour contg 0.05 ppm Co has proved satisfactory for this purpose.

6.033 CALCULATIONS

Express results in terms of ppm Co, based upon dry wt of sample.

(ml total soln/ml dithizone aliquot)
× (mmg Co/g dry sample) = ppm Co.

Value for mmg Co is obtained from curve minus ashing blank.

Nitroso-R-Salt Method (14)—First Action

6.034 REAGENTS

Those listed in **6.025** and following:

(**a**) *Nitroso-R-salt soln.*—0.2%. Dissolve 2 g powd. nitroso-R-salt (Eastman Kodak Co., No. 1124) in redistd H_2O, **6.025**(**a**), and dil. to 1 L.

(**b**) *Dilute nitric acid.*—(1+1). Dil. HNO_3 with equal vol. H_2O and redistill in Pyrex app. Store in Pyrex bottles.

(**c**) *Bromine water.*—Satd soln of Br in redistd H_2O, **6.025**(**a**).

(**d**) *Citric acid soln.*—0.2*N*. Use special reagent grade Pb-free citric acid.

6.035 PREPARATION AND ASHING OF SAMPLES

Proceed as in nitrosocresol method, **6.028**–**6.029**, thru "(Usually clear soln essentially free of insol. material is obtained.)" except use 10 g instead of 6 g dry plant tissue.

6.036 DITHIZONE EXTRACTION

Transfer entire soln to 120 ml separator, and proceed as in **6.030**, thru "If free acid remains . . . pH control is important." Dissolve in 1 ml of the citric acid soln, transfer to 25 ml vol. flask, and dil. to vol. with redistd H_2O, **6.025**(**a**).

6.037 DETERMINATION

Transfer suitable aliquot (ca 8 g dry material) of the citric acid soln, **6.036**, to 50 ml beaker. Evap. to 1–2 ml. Add 3 ml Na borate buffer, **6.025**(**n**), and adjust pH to 8.0–8.5 with NaOH (check externally with phenol red). (Vol. not >5 ml.) Add 1 ml of the nitroso-R-salt soln *slowly with mixing*. Boil 1–2 min. and add 2 ml of the dil. HNO_3. Boil 1–2 min., add 0.5–1.0 ml of the Br-H_2O, cover with watch glass, and let stand warm 5 min. Boil 2–3 min. to remove excess Br. Cool, and dil. to 10 or 25 ml (depending on length of light path in absorption cell). Transfer to absorption cell and read at 500 mμ within 1 hr. Prep. stds contg 0.5, 1, 2, 3, and 4 mmg Co and add 1 ml citric acid soln, **6.034**(**d**), to each. Proceed as for unknowns, beginning "Evap. to 1–2 ml."

Copper (14)—First Action

6.038 REAGENTS

Those listed in **6.025** and following:

(**a**) *Sodium diethyldithiocarbamate soln.*—0.1%. Freshly prepd in redistd H_2O, **6.025**(**a**).

(**b**) *Copper std soln.*—Dissolve 0.3930 g $CuSO_4.5H_2O$ in redistd H_2O, **6.025**(**a**), add 5 ml H_2SO_4, dil. to 1 L, and mix. Take 10 ml aliquot, add 5 ml H_2SO_4, dil. to 1 L, and mix. (1 ml = 1 mmg Cu.)

6.039 DETERMINATION

Transfer aliquot (0.5–1 g dry material) from soln obtained from **6.030** or **6.036** to 125 ml separator. Add 2 ml of the NH_4 citrate soln, 1 drop phthln, 5 ml of the Na diethyldithiocarbamate soln, and NH_4OH (1+1), **6.025**(**e**), until pink. Add 10 ml CCl_4 and shake 5 min. Drain the CCl_4, centrifuge 5 min., transfer to absorption cell, and read with filters (Corning) 3389 and 5113, or at 430 mμ.

Prep. std curve with 1, 5, 10, 15, and 20 mmg Cu treated as above.

Zinc

Mixed Color Method (15)—First Action

6.040 REAGENTS

(Redistill all H_2O from Pyrex glass. Treat all glassware with HNO_3(1+1) or fresh chromic acid cleaning soln. Rinse repeatedly with ordinary distd H_2O and finally with Zn-free H_2O.)

(**a**) *Carbon tetrachloride.*—Use ACS grade without purification. If tech. grade is used, dry with anhyd. $CaCl_2$ and redistill in presence of small quantity CaO. (Used CCl_4 may be reclaimed by distn in presence of NaOH (1+100) contg small quantities $Na_2S_2O_3$, followed by drying with anhyd. $CaCl_2$ and fractional distn in presence of small quantities CaO.)

(**b**) *Zinc std solns.*—(1) *Stock soln (1 mg Zn/ml).* Place 0.25 g pure Zn in 250 ml vol. flask. Add ca 50 ml H_2O and 1 ml H_2SO_4, and heat on steam bath until all Zn dissolves. Dil. to 250 ml and store in Pyrex vessel. (2) *Std soln (10 mmg Zn/ml).*—Dil. 10 ml stock soln to 1 L. Store in Pyrex vessel.

(**c**) *Ammonium hydroxide soln.*—1*N*. With all-Pyrex app. distill NH_4OH into H_2O, stopping distn when half has gone over. Dil. distillate to proper concn. Store in g-s. Pyrex vessel.

(**d**) *Hydrochloric acid.*—1*N*. Displace HCl gas from HCl in glass flask by slowly adding equal vol. H_2SO_4 from dropping funnel that extends below surface of the HCl. Absorb displaced HCl gas by conducting it thru delivery tube to surface of H_2O in receiving flask (no heat is necessary).

Dil. to proper concn. Use of 150 ml each of HCl and H_2SO_4 will yield 1 L purified HCl soln of concn $>1N$.

(**e**) *Diphenylthiocarbazone* (*dithizone*) *soln.*—Dissolve 0.20 g dithizone in 500 ml CCl_4, and filter to remove insol. matter. Place soln in g-s. bottle or large separator, add 2 L $0.02N$ NH_4OH (40 ml $1N$ NH_4OH dild to 2 L), and then shake to ext. dithizone into aq. phase. Sep. phases, discard CCl_4 phase, and ext. ammoniacal soln of dithizone with 100 ml portions CCl_4 until CCl_4 ext. is pure green. Discard CCl_4 phase after each extn. Add 500 ml CCl_4 and 45 ml of the $1N$ HCl, and shake to ext. the dithizone into the CCl_4. Sep. phases and discard aq. phase. Dil. CCl_4 soln of dithizone to 2 L with CCl_4. Store in brown bottle in dark, cool place.

(**f**) *Ammonium citrate soln.*—$0.5M$. Dissolve 226 g $(NH_4)_2HC_6H_5O_7$ in 2 L H_2O. Add NH_4OH (80–85 ml) until soln has pH of 8.5–8.7. Add excess of the dithizone soln (orange-yellow color in aq. phase after shaking and sepn of phases), and ext. with 100 ml portions CCl_4 until ext. is full green color. Add more dithizone if necessary. Sep. aq. phase from the CCl_4 and store in Pyrex vessel.

(**g**) *Carbamate soln.*—Dissolve 0.25 g Na diethyldithiocarbamate in H_2O and dil. to 100 ml with H_2O. Store in refrigerator in Pyrex bottle. Prep. fresh after 2 weeks.

(**h**) *Dilute hydrochloric acid.*—$0.02N$. Dil. 100 ml of the $1N$ HCl to 5 L.

6.041 PREPARATION OF SOLUTIONS

To reduce measuring out reagents and minimize errors due to variations in composition, prep. 3 solns in appropriate quantities from the reagents and store in Pyrex vessels, taking care to avoid loss of NH_3 from Solns A and B. Discard solns after 6–8 weeks because Zn increases slowly with storage. Det. std curve for each new set of reagents. Following quantities of Solns A and B and 2 L of the dithizone soln are enough for 100 detns:

(1) *Soln A.*—Dil. 1 L $0.5M$ NH_4 citrate and 140 ml $1N$ NH_4OH to 4 L.

(2) *Soln B.*—Dil. 1 L $0.5M$ NH_4 citrate and 300 ml $1N$ NH_4OH to 4.5 L. Just before using, add 1 vol. of the carbamate soln to 9 vols of the NH_3-NH_4 citrate soln to obtain vol. of Soln B immediately required.

NOTE: If Zn-free reagents have been prepd, they can be used to test chemicals for Zn. Certain lots of NH_4OH and HCl are sufficiently free of Zn to be used without purification.

6.042 ASHING

Ash 5 g finely ground, air-dried plant material in Pt dish in elec. muffle at 500–550°. Include blank detn. Moisten ash with little H_2O; then add 10 ml of the $1N$ HCl (more if necessary) and heat on steam bath until all substances sol. in HCl are dissolved. Add 5–10 ml hot H_2O. Filter off insol. matter on 7 cm paper (Whatman No. 42 or equiv. previously washed with two 5 ml portions hot $1N$ HCl, then washed with hot H_2O until free of HCl), and collect filtrate in 100 ml vol. flask. Wash filter with hot H_2O until washings are not acid to Me red. Add 1 drop Me red, **2.034**(i), to filtrate in 100 ml flask; neutralize with $1N$ NH_4OH and add 4 ml $1N$ HCl. Cool, and dil. to vol. with H_2O.

6.043 FIRST EXTRACTION

(Sepn of dithizone complex-forming metals from ash soln)

Pipet aliquot of ash soln contg not >30 mmg Zn into 125 ml Squibb separator. Add 1 ml $0.2N$ HCl for each 5 ml ash soln <10 ml taken, or 1 ml $0.2N$ NH_4OH for each 5 ml >10 ml taken. (10 ml aliquot is usually satisfactory in analysis of plant materials.) Add 40 ml Soln A and 10 ml of the dithizone reagent. Shake vigorously 30 sec. to ext. from aq. phase the Zn and other dithizone complex-forming metals that may be present; then let layers sep. At this point excess of dithizone (indicated by orange or yellow-orange color of aq. phase) must be present. If excess dithizone is not present, add more reagent until, after shaking, excess is indicated. Shake down the drop of CCl_4 ext. from surface, and drain CCl_4 ext. into second separator as completely as possible without letting any aq. layer enter stopcock bore. Rinse down CCl_4 ext. from surface of aq. layer with 1–2 ml clear CCl_4; then drain this CCl_4 into second separator without letting aq. phase enter stopcock bore. Repeat rinsing process as often as necessary to flush ext. completely into second separator. Add 5 ml clear CCl_4 to first separator, shake 30 sec., and let layers sep. (CCl_4 layer at this point will have clear green color if metals that form dithizone complexes have been completely extd from aq. phase by previous extn.) Drain CCl_4 layer into second separator and flush ext. down from surface and out of separator as directed previously. If last ext. does not possess distinct clear color, repeat extn with 5 ml clear CCl_4 and flushing-out process until complete extn of dithizone complex-forming metals is assured; then discard aq. phase.

6.044 SECOND EXTRACTION

(Sepn of Cu by extn of Zn into $0.02N$ HCl)

Pipet 50 ml of the $0.02N$ HCl into separator contg the CCl_4 soln of metal dithizonates. Shake vigorously 1.5 min., and let layers sep. Shake down drop from surface of aq. phase, and as completely as possible run off CCl_4 phase contg all Cu as dithizonate, without letting any of aq

phase, which contains all the Zn, enter stopcock bore. Rinse down CCl_4 ext. from surface of aq. phase, and rinse out stopcock bore with 1–2 ml portions clear CCl_4 (same as in first extn) until all traces of green dithizone have been washed out of separator. Shake down drop of CCl_4 from surface of aq. phase, and drain CCl_4 as completely as possible without letting any aq. phase enter stopcock bore. Remove stopper from separator and lay it across neck until small quantity of CCl_4 on surface of aq. phase evaps.

6.045 FINAL EXTRACTION

(Extn of Zn in presence of carbamate reagent)

Pipet 50 ml Soln B and 10 ml of the dithizone soln into 50 ml 0.02*N* HCl soln contg the Zn. Shake 1 min. and let phases sep. Flush out stopcock and stem of separator with ca 1 ml of the CCl_4 ext.; then collect remainder in test tube. Pipet 5 ml ext. into 25 ml vol. flask, dil. to mark with clear CCl_4, and det. transmittance of dild soln with photoelec. colorimeter equipped with Sextant Green (Corning No. 401) or equiv. filter, with max. transmission ca 525 mμ. (CAUTION: Protect final ext. from sunlight as much as possible and read within 2 hr.)

Det. Zn present in aliquot from curve relating transmittance and concn, correct for Zn in blank, and calc. % Zn in sample.

6.046 STANDARD CURVE

Obtain data for std curve by detg transmittance values for each of series of solns contg known quantities of Zn. To prep. these Zn solns, place 0, 5, 10, 15, 20, 25, 30, and 35 ml of the std Zn soln contg 10 mmg Zn/ml in 100 ml vol. flasks. To each flask add 1 drop Me red and neutralize with 1*N* NH_4OH; then add 4 ml 1*N* HCl and dil. to vol. Proceed exactly as for ash solns, beginning with first extn, and using 10 ml aliquots of each of the Zn solns. (The 10 ml aliquots contain 0, 5, 10, 15, 20, 25, 30, and 35 mmg Zn, resp.) Construct std curve by plotting mmg Zn against transmittance on semilog paper.

Single Color Method (16)—First Action

6.047 REAGENTS

See **6.040–6.041** plus following:

(**a**) *Dilute dithizone soln.*—Dil. 1 vol. dithizone soln, **6.040(e)**, with 4 vols CCl_4.

(**b**) *Carbamate soln.*—Dissolve 1.25 g Na diethyldithiocarbamate in H_2O and dil. to 1 L. Store in refrigerator and prep. fresh after long periods of storage.

(**c**) *Dilute ammonium hydroxide.*—Dil. 20 ml 1*N* NH_4OH, 6.040(c), to 2 L.

6.048 ASHING

Weigh 2 g sample finely ground plant material into suitable crucible (well-glazed porcelain, Vycor, or Pt), include crucible for blank detn, and heat in muffle at 500–550° until ashing is complete. Cool, moisten ash with little H_2O, add 10 ml 1*N* HCl (more if necessary to insure excess of acid), and heat on steam bath until all sol. material dissolves. Add few ml hot H_2O and filter thru quant. paper into 200 ml vol. flask. Wash paper with hot H_2O until washings are not acid to Me red. Add 2 drops Me red soln to filtrate, neutralize with 1*N* NH_4OH, add exactly 3.2 ml 1*N* HCl, dil. to vol. with H_2O, and mix.

6.049 FORMATION OF ZINC DITHIZONATE

(Removal of interferences and sepn of excess dithizone)

Pipet aliquot of ash soln contg not >15 mmg Zn into 125 ml amber glass separator. (25 ml aliquot is usually satisfactory.) If necessary to use different vol., add 0.4 ml 0.2*N* HCl for each 5 ml less, or 0.4 ml 0.2*N* NH_4OH for each 5 ml more than 25 ml taken. If <25 ml of the soln is taken, add H_2O to 25 ml.

Add 10 ml dithizone reagent, **6.040(e)**, to aliquot in separator and shake vigorously 1 min. Let layers sep. and discard CCl_4 layer. Add 2 ml CCl_4 to aq. soln, let layers sep., and discard the CCl_4. Repeat this rinsing once. Then add 5 ml CCl_4, shake vigorously 15 sec., let layers sep., and discard the CCl_4. Rinse once more with 2 ml CCl_4 as above. Discard CCl_4 layer and let CCl_4 remaining on surface of soln in funnel evap. before proceeding.

Add 40 ml of the NH_4 citrate Soln A, **6.041(1)**, 5 ml of the carbamate soln, **6.047(b)**, and 25 ml of the dil. dithizone reagent, **6.047(a)**. Add carbamate and dithizone reagents accurately from pipet or buret. Shake vigorously 1 min. Let layers sep. and draw off aq. layer thru fine tip glass tube connected to aspirator with rubber tubing. To remove excess dithizone from CCl_4 layer, add 50 ml 0.01*N* NH_4OH and shake vigorously 30 sec.

6.050 DETERMINATION

Dry funnel stem with pipestem cleaner and flush out with ca 2 ml of the Zn dithizonate soln. Collect adequate portion of remaining soln in 25 ml erlenmeyer, or other suitable container, and stopper tightly. (Amber glass containers are convenient, but colorless glassware will suffice if solns are kept in dark until transmittance readings are made.)

Measure transmittance of each soln against CCl_4 with photoelec. colorimeter equipped with light filter with max. transmittance near 535 mμ.

(Sextant Green Corning filter No. 4010 is suitable.) Correct for Zn in blank detns. Calc. quantity of Zn present in soln from curve relating concn and transmittance.

6.051 STANDARD CURVE

Into 200 ml vol. flasks place 0, 2, 4, 6, 8, 10, 12, and 14 ml portions, resp., of the std soln contg 10 mmg Zn/ml. To each flask add 2 drops Me red soln, neutralize with $1N$ NH_4OH, add 3.2 ml $1N$ HCl, and dil. to vol. with H_2O. Pipet 25 ml aliquots of each of these solns, contg 0, 2.5, 5, 7.5, 10, 12.5, 15, and 17.5 mmg Zn, resp., into amber glass separators, and proceed as for ash solns, **6.049**, second par., beginning "Add 10 ml dithizone reagent, **6.040(e)** . . . " Det. transmittance of each soln and plot values against corresponding quantities of Zn on semilog paper.

Molybdenum (*17*)—First Action

6.052 APPARATUS

Photoelectric colorimeter or spectrophotometer.—Capable of isolating band at ca 465 mμ. (Cenco-Sheard-Sanford Photelometer equipped with Corning filter No. 502 with max. transmittance at 440–460 mμ and 1 cm absorption cells of 10 ml capacity is suitable.)

6.053 REAGENTS

(**a**) *Amyl alcohol.*—Reagent grade iso-amyl alcohol (3-methyl-1-butanol), b.p. 128–132°.

(**b**) *Dilute hydrochloric acid.*—(1) *20% soln.*—Dil. concd HCl to ca 20% HCl. (2) *6N soln.*—Stdze to second decimal place.

(**c**) *Iron std soln.*—100 mmg Fe/ml. Dissolve 0.7022 g $Fe(NH_4)_2(SO_4)_2.6H_2O$ in H_2O, add 1 ml H_2SO_4, and dil. to 1 L.

(**d**) *Molybdenum std soln.*—Dissolve 0.0920 g $(NH_4)_6Mo_7O_{24}.4H_2O$ in H_2O and dil. to 500 ml. 1 ml = 100 mmg Mo. Prep. more dil. solns as required.

(**e**) *Potassium thiocyanate solution.*—20%. Dissolve 50 g KCNS in H_2O and dil. to 250 ml.

(**f**) *Sodium fluoride saturated soln.*— Add 200 ml H_2O to ca 10 g NaF. Stir until satd and filter.

(**g**) *Stannous chloride soln.*—(1) *20% soln.*—Weigh 10 g $SnCl_2.2H_2O$ into beaker, add 10 ml 20% HCl, (**b**)(1), and heat until completely dissolved. Cool, add granule of metallic Sn, dil. to 50 ml with H_2O, and store in g-s. bottle. (2) *0.8% wash soln.*—Dil. 4 ml of the 20% soln to 100 ml with H_2O.

6.054 DETERMINATION

(See Definitions of Terms and Explanatory Notes with regard to precautions on use of $HClO_4$)

Weigh 1–5 g of finely ground sample, contg not >35 mmg Mo, into 200 ml tall-form Pyrex beaker. To 1, 2, or 5 g samples add 10, 15, or 35 ml HNO_3, resp. Include 2 beakers for blanks. Cover beaker with cover glass, and let stand ca 15 min.; then heat cautiously on steam bath or hot plate at ca 100°, avoiding frothing over top. If froth approaches cover glass, remove beaker from heat until frothing subsides, then continue heating. Digest, usually ca 2 hr, until most of solids disappear.

Cool to room temp. If contents should go to dryness, add few ml HNO_3. Add 6 ml 70–72% $HClO_4$, cover beaker, place on hot plate, and gradually raise temp. so that contents boil vigorously but do not bump. Continue heating until digestion is complete as indicated by liquid becoming colorless or pale yellow. If necessary, make repeated additions of HNO_3 and $HClO_4$ and continue to digest until C is completely oxidized.

After digestion is complete, place cover glass slightly to one side of top of beaker, or replace it with Speedyvap cover glass or similar device, and evap. just to dryness or until residue appears only slightly moist. Remove beaker from hot plate, and cool. Wash down sides of beaker and underside of cover glass with few ml of H_2O, return to hot plate, and boil few min. Remove from hot plate, cool, and again rinse sides of beaker and cover glass with small amount of H_2O.

Add 2 drops Me orange and neutralize with NH_4OH. Add $6N$ HCl, dropwise with stirring, until soln is just acid; then add 8.2 ml excess to give final concn of ca 3% HCl. Add 2 ml satd NaF soln, and 1 ml Fe soln, if sample contains <100 mmg Fe.

Transfer soln to 125 ml separator and dil. to 50 ml with H_2O. Add 4 ml 20% KCNS soln, mix thoroly, and add 1.5 ml 20% $SnCl_2$ soln. Mix again, and from buret or pipet, add exactly 15 ml iso-amyl alcohol. Stopper funnel and shake vigorously 1 min., let phases sep., and draw off and discard aq. layer. Ext. into alcohol without delay, since colored complex is somewhat unstable in aq. soln.

Add 25 ml freshly prepd 0.8% $SnCl_2$ wash soln, and shake gently 15 sec. Let phases sep., and draw off and discard aq. layer. Transfer iso-amyl alcohol soln to tube of suitable size, and centrifuge 5 min. at ca 2000 rpm to remove H_2O droplets. If alcohol layer does not appear to be optically clear, recentrifuge. Stopper tubes to prevent evapn, if transmittance readings cannot be made immediately.

Compare unknown solns with iso-amyl alcohol at ca 465 mμ in a photoelectric colorimeter or spectrophotometer, and make appropriate corrections in transmittance readings for Mo in blanks. Obtain Mo concn from calibration curve relating transmittance (or absorbance) readings to concns of series of solns of known Mo content.

Prep. calibration curve for instrument used, as follows: Dil. 25 ml std Mo soln to 500 ml to obtain soln contg 5.0 mmg/ml. Place aliquots of this soln contg 0, 5, 10, 15, 20, 25, 30, and 35 mmg Mo, resp., into 200 ml tall-form beakers and carry them thru entire procedure, beginning with digestion with HNO_3 and $HClO_4$. Plot transmittance (or absorbance) readings against corresponding Mo concns.

NONMETALS

Arsenic—Official

6.055 PREPARATION OF SOLUTION—*See* **24.003**

6.056 DETERMINATION

Proceed as in **24.004–24.005**, or take aliquot and det. as in **4.009**, beginning "add 3 ml H_2SO_4 . . ."

Sulfur

Sodium Peroxide Method (18)—Official

6.057 PREPARATION OF SOLUTION

Place 1.5–2.5 g sample in ca 100 ml Ni crucible and add 5 g anhyd. Na_2CO_3. Mix thoroly, using Ni or Pt rod, and moisten with ca 2 ml H_2O. Add Na_2O_2, ca 0.5 g at time, thoroly mixing charge after each addn, and continue until mixt. becomes nearly dry and quite granular (ca 5 g Na_2O_2). Place crucible over S-free flame or elec. hot plate and heat carefully, stirring occasionally, until contents are fused. (If material ignites, detn is worthless.)

After fusion, remove crucible, let cool somewhat, and cover hardened mass with more Na_2O_2 to depth of ca 5 mm. Heat gradually and finally with full flame until fusion again takes place, rotating crucible occasionally to bring any particles adhering to sides into contact with oxidizing material. Continue heating 10 min. after fusion is complete. Cool somewhat, place warm crucible and contents in 600 ml beaker, and carefully add ca 100 ml H_2O. After initial violent action ceases, wash material out of crucible, make slightly acid with HCl (adding small portions at time), transfer to 500 ml vol. flask, cool, dil. to vol., and filter.

6.058 DETERMINATION

Dil. aliquot of prepd soln to ca 200 ml with H_2O and add HCl until ca 0.5 ml free acid is present. Heat to boiling and add 10 ml 10% $BaCl_2$ soln dropwise with constant stirring. Continue boiling ca 5 min., and let stand 5 hr or longer in warm place. Decant thru ashless paper or ignited and weighed gooch. Add 15–20 ml boiling H_2O to ppt, transfer to filter, and wash with boiling H_2O until filtrate is Cl-free. Dry ppt and filter, ignite, and weigh as $BaSO_4$. Wt ppt $\times 0.1374 = S$.

Magnesium Nitrate Method (19)—Official

6.059 PREPARATION OF SOLUTION

Weigh 1 g sample into large porcelain crucible. Add 7.5 ml $Mg(NO_3)_2$ soln, **2.017(e)**, so that all material comes in contact with soln. (It is important that enough $Mg(NO_3)_2$ soln be added to insure complete oxidation and fixation of S present. For larger samples and for samples with high S content, proportionally larger quantities of this soln must be used.) Heat on elec. hot plate (180°) until no further action occurs. Transfer crucible while hot to elec. muffle and let it remain at low heat (not >500°) until charge is thoroly oxidized. (No black particles should remain. If necessary, break up charge and return to muffle.) Remove crucible from muffle and let cool. Add H_2O; then HCl in excess. Bring soln to boil, filter, and wash thoroly. If preferred, transfer soln to 250 ml vol. flask before filtering and dil. to mark with H_2O.

6.060 DETERMINATION

Dil. entire filtered soln, **6.059,** to 200 ml, or take 100 ml aliquot of the measured vol., dil. to 200 ml, and proceed as in **6.058.**

Phosphorus (*20*)—Official

6.061 *Macro Method*

(**a**) *For samples exceedingly high in P and low in Ca and Mg (certain seeds, grains, etc.).*—Prep. soln as in **6.059,** or evap. filtrate and washings from S detn, **6.058,** to 50 ml, and proceed as in **2.019** or **2.022.**

(**b**) *For other samples.*—Take 50 ml aliquot of Soln A, **6.005,** and proceed as in **2.019** or **2.022.**

Micro Method (21)

6.062 REAGENTS

(**a**) *Phosphorus std soln.*—Dissolve 0.4394 g pure dry KH_2PO_4 in H_2O and dil. to 1 L. Dil. 50 ml of this soln to 200 ml (2 ml = 0.05 mg P).

(**b**) *Ammonium molybdate soln.*—Dissolve 25 g NH_4 molybdate in 300 ml H_2O. Dil. 75 ml H_2SO_4 to 200 ml and add to NH_4 molybdate soln.

(**c**) *Hydroquinone soln.*—Dissolve 0.5 g hydroquinone in 100 ml H_2O, and add 1 drop H_2SO_4 to retard oxidation.

(**d**) *Sodium sulfite soln.*—Dissolve 200 g Na_2SO_3 in H_2O, dil. to 1 L, and filter. Either keep this soln well stoppered or prep. fresh each time.

6.063 PREPARATION OF SOLUTION

To 1 or 2 g sample in small porcelain crucible add 1 ml $Mg(NO_3)_2$ soln, **2.017(e)**, and place on

steam bath. After few min. cautiously add few drops HCl, taking care that gas evolution does not push portions of sample over edge of crucible. Make 2 or 3 further addns of few drops HCl while sample is on bath so that as it approaches dryness it tends to char. If contents of crucible become too viscous for further drying on bath, complete drying on hot plate. Cover crucible, transfer to cold muffle, and ignite 6 hr at 500°, or until even gray ash is obtained. (If necessary, cool crucible, dissolve ash in little H_2O or alc.-glycerol, evap. to dryness, and return uncovered to muffle 4–5 hr longer.) Cool, take up with HCl (1+4), and transfer to 100 ml beaker. Add 5 ml HCl and evap. to dryness on steam bath to dehydrate SiO_2. Moisten residue with 2 ml HCl, add ca 50 ml H_2O, and heat few min. on bath. Transfer to 100 ml vol. flask, cool immediately, dil. to vol., and filter, discarding first portion of filtrate.

6.064 DETERMINATION

To 5 ml aliquot filtrate in 10 ml vol. flask add 1 ml of the NH_4 molybdate soln, rotate flask to mix, and let stand few sec. Add 1 ml of the hydroquinone soln, again rotate flask, and add 1 ml of the Na_2SO_3 soln. (Last 3 addns may be made with Mohr pipet.) Dil. to vol. with H_2O, stopper flask with thumb or forefinger, and shake to mix contents thoroly. Let stand 30 min., and compare immediately in colorimeter with 2 ml of the std KH_2PO_4 soln treated simultaneously and identically. (With either unknown or std set at 25.0 mm, readings within 10 mm, *i.e.*, range of 20 mm, are accurate. If concn of P in unknown set is outside this range, it may be brought nearer to that of std by dilg filtrate, ashing smaller or larger sample, making filtrate to smaller or larger vol., or using smaller aliquot. Photoelec. colorimeter equipped with filter with max. transmittance of 625–675 mμ may be used instead of visual instrument.) Report as % P.

Chlorine (*22*)—Official

(If bromides or iodides are present in significant quantities, correct results accordingly.)

6.065 PREPARATION OF SOLUTION

First verify complete retention of Cl in each kind of material by trial, since losses can occur, especially with samples high in carbohydrates, if insufficient Na_2CO_3 is present during ignition, or in any case if excessive temp. is used.

Moisten 5 g sample in Pt dish with 20 ml 5% Na_2CO_3 soln, evap. to dryness, and ignite as thoroly as possible at not >500°. Ext. with hot H_2O, filter, and wash. Return residue to Pt dish and ignite to ash; dissolve in HNO_3 (1+4), filter, wash thoroly, and add this soln to H_2O ext.

6.066 *Gravimetric Method*

To prepd soln, **6.065**, add 10% $AgNO_3$ soln, avoiding more than slight excess. Heat to boiling, protect from light, and let stand until ppt coagulates. Filter on weighed gooch, previously heated to 140–150°, and wash with hot H_2O, testing filtrate to prove excess of $AgNO_3$. Dry AgCl at 140–150°, cool, and weigh. Report as % Cl.

Volumetric Method I. (*23*)

(Since limit of accuracy of this titrn is considered to be ±0.2 mg Cl, accuracy of 1.0% requires samples contg not <20 mg.)

6.067 REAGENTS

(**a**) *Silver nitrate std soln.*—Prep. soln slightly stronger than 0.1*N*, stdze as in **42.029**, and adjust to exactly 0.1*N*. 1 ml 0.1*N* $AgNO_3$ = 0.00355 g Cl.

(**b**) *Ammonium or potassium thiocyanate std soln.*—0.1*N*. Prep. soln slightly stronger than 0.1*N*, stdze as in **42.028**(**b**), and adjust to exactly 0.1*N*.

(**c**) *Ferric indicator.*—Satd soln of $FeNH_4(SO_4)_2 . 12H_2O$.

(**d**) *Nitric acid.*—Free from lower oxides of N by dilg usual pure acid with ca ¼ vol. H_2O, and boiling until perfectly colorless.

6.068 DETERMINATION

To prepd soln, **6.065**, add known vol. of the $AgNO_3$ soln in slight excess. Stir well, filter, and wash AgCl ppt thoroly. To combined filtrate and washings add 5 ml of the ferric indicator and few ml of the HNO_3, and titr. excess Ag with the thiocyanate to permanent light brown. From ml $AgNO_3$ used calc. quantity of Cl.

Volumetric Method II. (*24*)

6.069 REAGENTS

(**a**) *Potassium iodide std soln.*—Weigh 4.6822 g pure (ACS) KI, dried to constant wt at 105–150°, dissolve in H_2O, and dil. to 1 L. 1 ml = 1 mg Cl.

(**b**) *Silver nitrate stock soln.*—Approx. 0.3*N*. Dissolve 48 g $AgNO_3$ in H_2O, filter, and dil. to 1 L. 1 ml = ca 10 mg Cl.

(**c**) *Silver nitrate std soln.*—Dil. 100 ml reagent (**b**) to ca 900 ml and adjust by stdzg against reagent (**a**) so that 1 ml = 1 mg Cl.

(**d**) *Chloride-free starch indicator.*—For each 100 ml final soln take 2.5 g sol. starch and make to paste with cold H_2O. Stir out lumps, add 25–50 ml more cold H_2O, and stir or shake 5 min. Centrifuge, decant, and discard liquid. Repeat extn 3 times and finally transfer residue to flask contg proper quantity boiling H_2O. Stir again, heat to boil, cover with small beaker, and cool under tap, shaking occasionally.

(e) *Dilute sulfuric acid.*—Add 35 ml H_2SO_4 to each 1 L H_2O, boil 5–10 min., and cool to room temp.

(f) *Iodine indicator.*—To ca 20 g I in 500 ml g-s. bottle add 400 ml dil. H_2SO_4, (e), and shake 10 min. Decant and discard first soln, since it may contain iodides. Repeat process and store soln in small g-s. bottles.

(g) *Potassium permanganate soln.*—Dissolve 60 g $KMnO_4$ in 400 ml warm H_2O (ca 50°) and dil. to 1 L.

(h) *Potassium sulfate-copper sulfate mixture.*—Thoroly mix 16 parts K_2SO_4 and 1 part $CuSO_4.5H_2O$.

(i) *Wash soln.*—Mix 980 ml H_2O and 20 ml HNO_3.

6.070 DETERMINATION

Weigh into beaker sample contg 10–40 mg Cl. (If >4 g is taken, use proportionately more HNO_3 and $KMnO_4$ soln.) Add 10 ml of the 0.3*N* $AgNO_3$ and stir until sample is thoroly soaked with the soln, adding little H_2O or warming if necessary. Add 25 ml HNO_3, stir, add 5 ml of the $KMnO_4$ soln, and stir until frothing stops. Place mixt. in H_2O bath or on hot plate and keep just below boiling. Stir, and wash down sides of beaker at intervals with min. quantity of H_2O. After 20 min., or when reaction stops, add more of the $KMnO_4$ soln, little at time, until color begins to fade slowly. Dil. to ca 125 ml with boiling H_2O and heat 10 min. longer. (Beaker may stand in bath or on hot plate until ready to filter.)

Filter while hot thru Whatman No. 5, or equiv. paper, with suction as follows: Place disk of 30-mesh stainless steel wire gauze or No. 40 filter cloth in bottom of 3″ Hirsch funnel. Fold 9 cm paper over botton of No. 11 rubber stopper, shaping it to funnel by making 9–10 folds up side of stopper. Place paper in funnel and apply strong suction. Wet paper and keep wet while fitting into funnel so as to avoid double thicknesses of paper. Wash paper thoroly, first with H_2O and then with the wash soln. Discard washings and rinse out flask. Decant thru filter and transfer ppt and sample residue to filter. If filtrate is not turbid, or if it is only slightly opalescent, wash ppt thoroly, applying wash soln very gently, but keeping strong suction on filter. If combined filtrate and washings are clear, test for Ag. If turbid, re-heat and pass thru filter, repeating until clear, and finally wash as above. If filtrate does not give definite test for Ag, repeat detn on smaller sample.

Place paper and contents in Kjeldahl flask and add such quantities of the K_2SO_4-$CuSO_4$ mixt. and H_2SO_4 as would be appropriate for protein detn on same kind and quantity of sample, and digest similarly. (For 2 g grass, 8 g of the sulfate mixt. and 20 ml acid are enough.) When digest is cool, add 175 ml H_2O, boil 5–10 min., and cool to room temp. Titr. the Ag_2SO_4 in the Kjeldahl flask with the std KI, using 5 ml of the starch indicator and 30 ml of the I indicator. (Add latter just before titrn.) Rinse neck of flask after each addn of KI when near end point and titr. until blue color persists after shaking. If <30 mg Cl is present, add the starch and I solns at beginning. If larger but unknown quantity is present, add 2 ml starch and 10 ml I indicator at beginning and titr. until approach of end point is seen. Shake vigorously to coagulate ppt, add rest of starch and I solns, and proceed to end point. If known large quantity is present, titr. to within 2 ml of end point, shake as above, add indicator reagents, and continue titrn. If end point is overrun, add 5 ml of the std $AgNO_3$ soln and titr. again.

Blank detns are not necessary after testing reagents. If blanks made by using pure sugar as sample is >0.05 mg, examine filter paper, distd H_2O, and various reagents for Cl.

6.071 Selenium (*25*)—Official

(Applicable to materials contg >2 ppm Se)

Grind air-dried sample and carefully prep. uniform subsample. Prep. mixt. of 50 ml H_2SO_4 and 100 ml HNO_3 in 600 ml beaker. Add 5 g powd. sample to acid mixt. slowly, with stirring, restricting temp. of mixt. to not >80°. When first vigorous reaction is over, warm gently with occasional stirring until evolution of NO_2 fumes ceases. Warm at temp. not >120° until liquid darkens slightly. Transfer liquid to distg flask equipped with short condenser, thistle safety tube, and ⫪ connections, Fig. 15. Add 100 ml *HBr contg 2 ml Br*. Warm gently 15 min. and distill into 100 ml erlenmeyer contg 5 ml H_2O. Have outlet of distg tube submerged. If, on gentle warming, drop of Br does not collect beneath H_2O in receiver, add 2 ml Br to distg flask thru thistle tube and repeat gentle warming. Distill 60 ml into receiver. To distillate add 25 ml H_2O and cool in ice-H_2O. Pass slow stream of *SO_2* into distillate until Br is removed. Add 0.25 g *$NH_2OH.HCl$*, warm on steam bath 15 min. at 80°, and let stand overnight at room temp. Se appears at bottom of erlenmeyer as rose-pink ppt. Modify further treatment according to quantity of ppt.

(a) *Precipitate not greater than 0.5 mg.*—Filter Se ppt thru small asbestos gooch with suction. If small quantity of oily material accompanies pptd Se, wash pad with 10 ml alcohol and then with 10 ml H_2O. Redissolve pptd Se from pad with 10 ml 48% HBr which has been rendered bright red by addn of Br. Collect filtrate by suction in 25 ml vol. flask and wash pad with 2 portions H_2O. Decolorize filtered soln with SO_2 and add 1 ml soln contg 100 mg $NH_2OH.HCl$ and 25 mg *gum arabic*/ml. Dil. to vol. with H_2O. Transfer

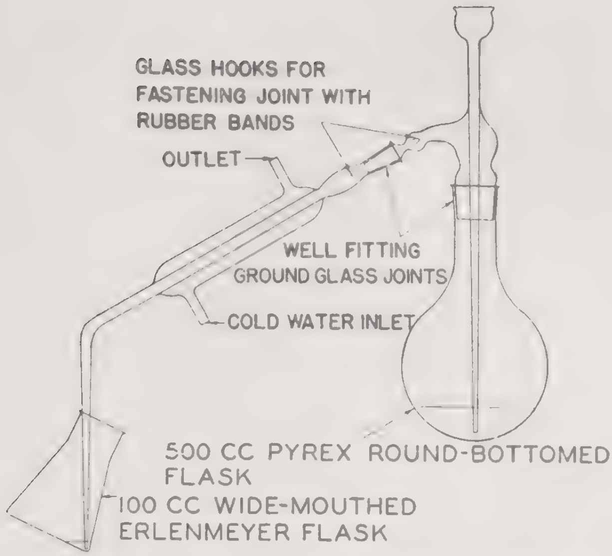

FIG. 15.—APPARATUS FOR DISTILLATION OF SELENIUM

flask and contents to steam bath and heat 30 min. at 80°; cool to room temp., shake vigorously, and transfer to 50 ml Nessler tube.

Before final pptn of the Se in vol. flask, prep. series of stds in 25 ml vol. flasks by addn of 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, and 0.7 mg Se as Na_2SeO_4. Ppt these stds after addn of HBr, Br, H_2O, $NH_2OH.HCl$, and gum arabic, and treat precisely as sample is treated. Compare sample with std in any suitable color comparator and det. quantity of Se. Express results as ppm air-dried sample.

(b) *Precipitate below 1 ppm (established by preceding determination) and for greater precision.*—Distill 10 g sample with HBr, as above, after preliminary acid treatment. When distn is complete, replace sample in distn flask with second acid-digested 10 g sample; add distillate from first distn, 50 ml addnl HBr and 2–4 ml Br, and 22 ml H_2SO_4. Let stand and repeat distn as often as necessary to integrate minute quantities of Se until quantity adequate for measurement is obtained.

(c) *Initial precipitate in excess of 0.5 mg.*—Redissolve washed ppt in HBr, colored with Br as in (a). Transfer dissolved material to 100 ml beaker and dil. with 20% HBr to 50 ml. Ppt with SO_2 and add 0.25 g $NH_2OH.HCl$. Warm on steam bath 15 min., and let stand overnight at room temp. Filter on weighed gooch, dry 4 hr at 85°, and weigh. (Use balance sensitive to at least 0.05 mg.)

Boron (*26*)—First Action

Quinalizarin Method

6.072 REAGENTS

(a) *Dilute sulfuric acid.*—0.36*N*. Dil. 10 ml H_2SO_4 to 1 L.

(b) *Calcium hydroxide satd soln.*— Filter before use.

(c) *Quinalizarin soln.*—Dissolve 45 mg quinalizarin in 1 L 95–96% H_2SO_4.

(d) *Boron std soln.*—2.857 g H_3BO_3/L H_2O (1 ml = 500 mmg B). Prep. working stds by further diln with H_2O.

6.073 DETERMINATION

Place 1.00–2.00 g dry, ground plant material in Pt or SiO_2 dish. Add 5 ml satd $Ca(OH)_2$ soln and dry at 105°. Carefully drive off volatile material over burner, ash in muffle 1 hr at 600°, and cool. Add exactly 10 or 15 ml 0.36*N* H_2SO_4, break up ash with glass rod, stir gently, and filter. Transfer 2 ml filtrate to colorimeter tube, add an exact quantity (e.g., 15 ml) quinalizarin reagent, stopper tube, and mix by whirling gently. Let tube stand at room temp. 24 hr (or until both unknowns and stds have cooled to same temp). Shake tube again immediately before reading in photoelec. colorimeter (620 mμ filter).

Adjust colorimeter to 100% transmittance with blank soln prepd as above but using 2 ml H_2O in place of sample soln. Prep. std curve with series of stds contg 0.5 to 10 mmg B/ml.

OTHER CONSTITUENTS

Sugars (*27*)

6.074 PREPARATION OF SOLUTION—OFFICIAL

(a) *General method.*—Prep. fresh sample as in 6.002(b). Pour alc. soln thru filter paper or extn thimble, catching filtrate in vol. flask. Transfer insol. material to beaker, cover with 80% alcohol, warm on steam bath 1 hr, let cool, and again pour alc. soln thru same filter. If second filtrate is highly colored, repeat extn. Transfer residue to

filter, let drain, and dry. Grind residue so that all particles will pass thru 1 mm sieve, transfer to extn thimble, and ext. 12 hr in Soxhlet app. with 80% alcohol. Dry residue and save for starch detn. Combine alc. filtrates and dil. to vol. at definite temp. with 80% alcohol.

For dried materials, grind samples finely, and mix well. Weigh sample into beaker, and continue as above, beginning "cover with 80% alcohol . . ."

(**b**) *Applicable when starch is not to be determined.*—Prep. fresh sample as in **6.002(b)**, but boil on steam bath 1 hr. Decant soln into vol. flask, and comminute solids in high speed blender with 80% alcohol. Boil blended material on steam bath 0.5 hr, cool, transfer to vol. flask, dil. to mark with 80% alcohol at room temp., filter, and take aliquot for analysis.

Grind dry material to pass 20 mesh or finer, transfer weighed sample to vol. flask, and add 80% alcohol and enough $CaCO_3$ to neutralize any acidity. Boil 1 hr on steam bath, cool, adjust vol. at room temp. with 80% alcohol, filter, and take aliquot for analysis.

6.075 CLARIFICATION WITH LEAD—OFFICIAL

Place aliquot of the alc. ext. in beaker on steam bath and evap. off alcohol. Avoid evapn to dryness by adding H_2O if necessary. When odor of alcohol disappears, add ca 100 ml H_2O and heat to 80° to soften gummy ppts and break up insol. masses. Cool to room temp. and proceed as in (**a**) or (**b**):

(**a**) Transfer soln to vol. flask, rinse beaker thoroly with H_2O, and add rinsings to flask. Add enough satd neutral $Pb(OAc)_2$ soln to produce flocculent ppt, shake thoroly, and let stand 15 min. Test supernatant with few drops of the $Pb(OAc)_2$ soln. If more ppt forms, shake and let stand again; if no further ppt forms, dil. to mark with H_2O, mix thoroly, and filter thru dry paper. Add enough solid Na oxalate to filtrate to ppt all the Pb, and refilter thru dry paper. Test filtrate for presence of Pb with little solid Na oxalate.

(**b**) Add double min. quantity of satd neutral $Pb(OAc)_2$ soln required to cause complete pptn, as found by testing portion of supernatant with few drops dil. Na oxalate soln. After letting mixt. stand only few min., filter into beaker contg estimated excess of Na oxalate crystals. Let Pb ppt drain on filter and wash with cold H_2O until filtrate no longer gives ppt in oxalate soln. Assure excess of oxalate by testing with drop of the $Pb(OAc)_2$. Filter off and wash pptd Pb oxalate, catching filtrate and washings in vol. flask. Dil. to mark with H_2O and mix.

6.076 CLARIFICATION WITH ION-EXCHANGE RESINS (*28*)—FIRST ACTION

Place aliquot of the alc. ext., **6.074**, in beaker and heat on steam bath to evap. off alcohol. Avoid evapn to dryness by adding H_2O. When odor of alcohol disappears, add ca 15–25 ml H_2O and heat to 80° to soften gummy ppts and break up insol. masses. Cool to room temp. Prep. thin mat of Celite on filter paper in büchner or on fritted glass filter and wash until H_2O comes thru clear. Filter sample thru Celite mat, wash mat with H_2O, dil. filtrate and washings to appropriate vol. in vol. flask, and mix well.

Place 50.0 ml aliquot in 250 ml erlenmeyer; add 2 g *Amberlite IR-120(H)* analytical grade cation and 3 g *Duolite A-4(OH)* anion ion-exchange resins. Let stand 2 hr with occasional swirling. Take 5 ml aliquot de-ionized soln and det. reducing sugars as dextrose as in **29.055–29.057.**

Dextrose

6.077 *Micro Method—First Action—See* **29.056**

Fructose (*29*)—First Action

6.078 REAGENTS

(**a**) *Glucose oxidase prepn.*—Add slowly, stirring constantly, 100 ml H_2O to 5 g glucose oxidase prepn ("Dee-O," Takamine Laboratories, Clifton, N. J.). Stir ca 1 min. and centrifuge or filter to obtain clear soln. Add ca 1 ml $CHCl_3$ and refrigerate. This soln is stable at least 1 month.

(**b**) *McIlvaine's citrate-phosphate buffer.*—Dissolve 214.902 g $Na_2HPO_4.12H_2O$ and 42.020 g citric acid in H_2O and dil. to 1 L.

6.079 DETERMINATION

To suitable aliquot add ¼ its vol. of buffer to give pH ca 5.8. Add 30% as much glucose oxidase prepn as estimated glucose content (for 500 mg glucose add 150 mg glucose oxidase, *i.e.*, 3 ml soln), and few drops 30% H_2O_2 (omit if Somogyi method is to be used in detn). Let stand overnight at room temp.

Determine fructose by Somogyi micro method, **29.056,** or by Munson-Walker method, **29.039–29.040,** using table below. Check equivs in range of interest, using pure fructose as std and make appropriate corrections.

Abbreviated Munson and Walker Table for Calculating Fructose

(*From Official and Tentative Methods of Analysis, AOAC, 5th Ed., 1940*)

CUPROUS OXIDE MG	FRUCTOSE MG	CUPROUS OXIDE MG	FRUCTOSE MG
10	4.5	300	148.6
50	23.5	350	174.9
100	47.7	400	201.8
150	72.2	450	229.2
200	97.2	490	253.9
250	122.7	—	—

Reducing Sugars—Official

6.080 *Munson-Walker General Method*—*See* **29.039**

6.081 *Quisumbing-Thomas Method*—*See* **29.050**

Sucrose—Official

6.082 *Hydrochloric Acid Inversion*

Using aliquot of cleared soln obtained in **6.075**, proceed as in **22.042**.

6.083 *Invertase Inversion*

(1) *For plants giving hydrolysis end point within 2 hours.*—Pipet aliquot of cleared soln, **6.075**, into 400 ml Pyrex beaker and make slightly acid to Me red with HOAc. Add 3 drops 1% soln of *Wallerstein red label invertase.* Let mixt. stand at room temp. 2 hr. Add reagents as in **29.050** or **29.036**, and det. reducing power. Calc. results as invert sugar. Deduct reducing power of original soln, also expressed as invert sugar, and multiply difference by 0.95.

(2) *For plants giving slower hydrolysis end point.*—Place aliquot of soln, **6.075**, in small vol. flask. Make slightly acid to Me red with HOAc. Add 3 drops 1% soln of Wallerstein red label invertase and few drops toluene. Stopper flask and let stand overnight or longer at room temp. Dil. to mark with H_2O and use aliquot for reducing power as above. Results may include some other carbohydrates slowly hydrolyzed by invertase.

6.084 **Ether Extract—Official**—*See* **22.033**

6.085 **Crude Fiber—Official**—*See* **22.040**

6.086 **Total Nitrogen—Official**—*See* **2.037**

6.087 **Nitrogen (Nitrate-Free Samples)—Official**—*See* **2.036**

Starch (*30*)—First Action

6.088 REAGENTS

(**a**) *Iodine-potassium iodide soln.*—Grind 7.5 g I and 7.5 g KI with 150 ml H_2O, dil. to 250 ml, and filter.

(**b**) *Alcoholic sodium chloride soln.*—Mix 350 ml alcohol, 80 ml H_2O, and 50 ml 20% NaCl soln, and dil. to 500 ml with H_2O.

(**c**) *Alcoholic sodium hydroxide soln.*—0.25*N*. Mix 350 ml alcohol, 100 ml H_2O, and 25 ml 5*N* NaOH, and dil. to 500 ml with H_2O.

(**d**) *Dilute hydrochloric acid.*—0.7*N*. Dil. 60 ml HCl to 1 L with H_2O.

(**e**) *Somogyi's phosphate sugar reagent.*—Dissolve 56 g anhyd. Na_2HPO_4 and 80 g Rochelle salt in ca 1 L H_2O, and add 200 ml 1.00*N* NaOH. Then add slowly, with stirring, 160 ml 10% $CuSO_4.5H_2O$ soln. Dissolve 360 g Na_2SO_4 in this soln, transfer to 2 L vol. flask, and add exactly 200 ml 0.1*N* KIO_3 soln (3.5760 g/L). Dil. to vol., mix well, let stand several days, and filter thru dry paper into dry flask, discarding first 50 ml filtrate. Store reagent at 20–25°. It is 0.01*N* with respect to KIO_3; 5.00 ml is equiv. to 10 ml 0.005*N* $Na_2S_2O_3$.

Det. glucose factor of reagent as follows: Weigh accurately 150 mg NBS glucose into 1 L vol. flask, dissolve in H_2O, dil. to vol., and mix well. Transfer 5 ml aliquot to 25×200 mm Pyrex test tube, add exactly 5 ml of the Somogyi reagent, stopper with size 00 crucible, and heat (together with several blanks contg 5 ml H_2O and 5 ml reagent) exactly 15 min. in boiling H_2O bath. Titr. as in detn. From difference between blank and std titrns, calc. mg glucose equiv. to 1 ml exactly 0.005*N* $Na_2S_2O_3$. Effective range for detn is 0.05–1.0 mg glucose in 5 ml aliquot.

(**f**) *Sodium thiosulfate std soln.*—0.005*N*. Dissolve 2.73 g $Na_2S_2O_3.5H_2O$ in H_2O and dil. to 2 L. Stdze daily as follows: Add 1 ml KI soln, (**g**), and 3 ml 1.5*N* H_2SO_4 to 5 ml of the Somogyi sugar reagent. Let stand 5 min., and titr. with the $Na_2S_2O_3$ soln, adding starch indicator, **32.037(b)**, just before end point.

(**g**) *Potassium iodide soln.*—2.5%. Stabilize with little Na_2CO_3.

6.089 DETERMINATION

Select sample as in **6.001**, remove all foreign matter, dry, and grind to pass 80 mesh. Weigh accurately 0.1–1.0 g powd. sample contg ca 20 mg starch into Pyrex test tube, 25×150 mm. Add ca 200 mg fine sand and 5 ml H_2O, and mix well with stirring rod to wet sample. Heat tube in boiling H_2O bath 15 min. to gelatinize starch. Cool to room temp., and place in 22–25° bath. Add 5 ml 60% $HClO_4$ rapidly with constant agitation. Grind tissue against lower wall of tube with stirring rod for ca min. at time. Repeat grinding frequently during 30 min.; then without delay transfer quantitatively to 100 ml vol. flask with H_2O. Add 3 ml 5% uranyl acetate soln to ppt protein, dil. to vol. with H_2O, mix well, and centrifuge portion of mixt. Pipet 10 ml clear supernatant into 25×150 mm test tube. Add ca 100 mg Celite, 5 ml 20% NaCl soln, and 2 ml of the I-KI reagent, and mix well. Let stand overnight, centrifuge, and decant supernatant.

Wash starch-I ppt by suspending it in 5 ml of the alc. NaCl soln, centrifuge, and decant supernatant. Add 2 ml of the alc. NaOH soln to packed ppt. Gently shake and tap tube until blue color is discharged. (Do not use stirring rod; allow ample time for complex to decompose.) Wash walls of tube with 5 ml of the alc. NaCl soln, centrifuge liberated starch, and wash with 5 ml of the alc. NaCl soln as before. Add 2 ml of the 0.7*N* HCl to ppt. Stopper tube loosely with size

00 crucible, and heat 2.5 hr in boiling H_2O bath. (Provide bath with cover with holes to accommodate tubes. It is important that holes not occupied by tubes be covered.) Cool, and transfer quantitatively to 25 ml vol. flask. Add drop phenol red, **13.022**, and neutralize with $1N$ NaOH. Discharge color with *0.1N oxalic acid*, dil. to vol., and mix well. Transfer 5 ml aliquot to 25×200 ml Pyrex test tube, add exactly 5 ml of the Somogyi reagent, and stopper tube with size 00 crucible. Heat together with several blanks contg 5 ml H_2O and 5 ml of the Somogyi reagent in vigorously boiling H_2O bath exactly 15 min. Remove tube from bath and cool to 25–30°. Add 1 ml of the 2.5% KI soln down wall of tube without agitation and then add 3 ml $1.5N$ H_2SO_4 rapidly with agitation. After all Cu_2O dissolves, titr soln with $0.005N$ $Na_2S_2O_3$, adding starch indicator, **32.037(b)**, just before end point is reached. Treat blank solns similarly.

$$\% \text{ starch} = [50(\text{ml blank} - \text{ml sample}) \times 0.90/\text{mg sample}] \times (N/0.005) \times G \times 100$$

where 50 = diln factor, 0.90 = factor glucose to starch, N = actual normality $Na_2S_2O_3$ soln, and G = mg glucose equiv. to 1 ml $0.005N$ $Na_2S_2O_3$.

Lignin (*31*)—First Action

Direct Method

6.090 PREPARATION OF SAMPLE

Grind sample in mill to pass No. 80 sieve and dry at 105°. Ext. weighed sample (5–10 g) 30 hr in Soxhlet app. with alcohol-benzene soln (32 parts alcohol and 68 parts benzene by wt). Dry material in oven to free it from solvents and place in flask of suitable size. Add 150 ml H_2O/1 g sample, and boil mixt. under reflux condenser 3 hr. Filter mixt. while still hot, preferably thru weighed fritted glass crucible, and transfer extd material to flask. Add 1% HCl (111 g concd HCl+3890 ml H_2O) in proportion of 150 ml acid soln/1 g plant material, and boil under reflux condenser 3 hr. Filter mixt. while still hot thru fritted glass crucible previously used, wash with H_2O until acid-free, dry at 105°, and weigh. Calc. % total loss due to successive extn with the alcohol-benzene soln, hot H_2O, and the 1% HCl. (With samples not especially rich in carbohydrates and proteins, extn with hot H_2O may be omitted.)

6.091 APPARATUS

App., Fig. 16, consists of: (1) 1500 ml bottle, A, to which is attached by 2-hole rubber stopper 250 ml dropping funnel, C, having lower end of stem bent as illustrated and placed close to bottom of A; (2) Drechsel gas-washing bottle, D; (3) 3 Pyrex test tubes, 38×300 mm diam., G, G', G'', connected in parallel by device, O, and immersed in wooden box, L, filled with crushed ice, H; and (4) bottle contg H_2O for absorption of excess HCl, K. G, G', and G'' are provided with 2-hole rubber stoppers; glass tube with right angle bend extends thru 1 hole nearly to bottom of test tube, and similar tube extending ca 10 mm into test tube passes thru other hole. Rubber connec-

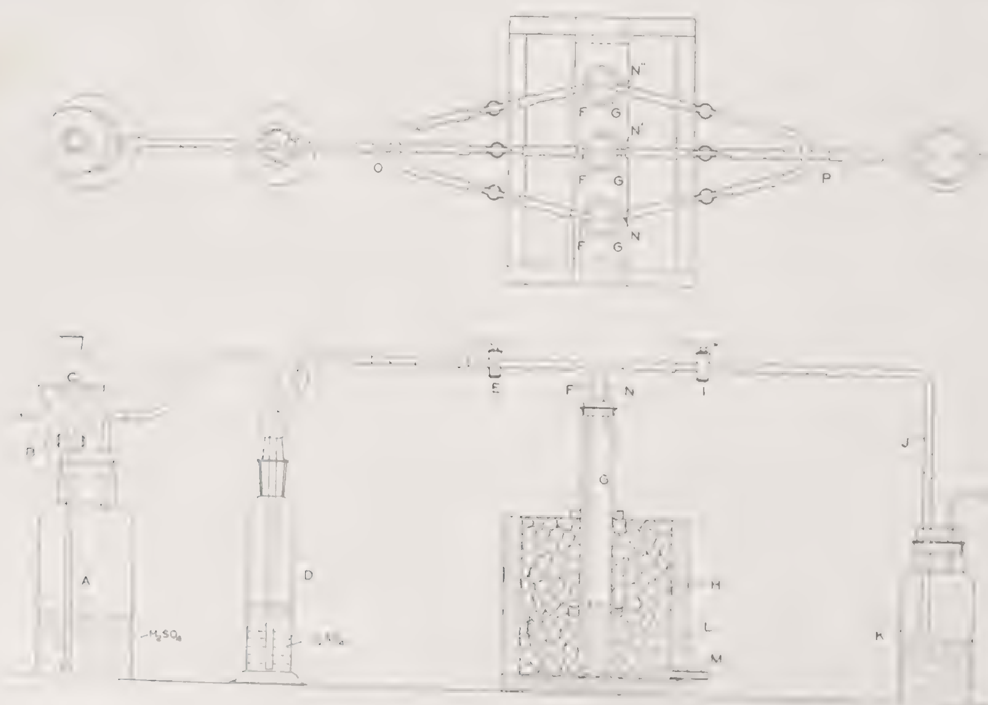

FIG. 16.—APPARATUS FOR DETERMINATION OF LIGNIN

tions and stopcocks for regulating flow of gas are provided as indicated in diagram. *A* is filled with ca 500 ml H_2SO_4 and *C* with HCl; HCl flowing thru stopcock *B* into *A* generates HCl gas, which is dried by H_2SO_4 in *D*, and flows into *G*, *G′*, and *G″* contg samples and fuming HCl reagent.

6.092 REAGENT

Fuming hydrochloric acid.—Density 1.212–1.223 at 15°. To 500 g NaCl in 1 L g-s. Pyrex distg flask, add cold soln of 250 ml H_2O in 450 ml H_2SO_4. Connect side tube of distg flask to glass tube passing thru H_2SO_4 wash bottle, and connect outlet tube of H_2SO_4 wash bottle to another glass tube, immersed in flask contg 3 L HCl. Surround flask contg the HCl with crushed ice. Heat distg flask with small flame and pass the HCl gas into the acid soln until it attains sp. gr. of 1.212–1.223 at 15°. Keep reagent refrigerated at 0° or below. If only few detns are to be made, prep. correspondingly smaller quantity.

6.093 DETERMINATION

Weigh three 1 g samples of extd and dried sample in weighing bottle and place in the 3 large test tubes, *G*, *G′*, and *G″*. Add 20 ml of the reagent to each tube, using this acid to wash down any particles clinging to sides. When all material is wet with reagent, add 30 ml more of the reagent. Add ca 3 drops *capryl alcohol* to minimize foaming. Place 3 large test tubes, *G*, *G′*, and *G″*, in wooden box, *L*, and surround with crushed ice. Lubricate tubes *F*, *F′*, and *F″* with drop of glycerol so that they move easily thru holes in rubber stoppers. Lead dry HCl gas from generator into reaction mixts thru tubes *F*, *F′*, and *F″* (*F′* and *F″* are shown in top view), which reach nearly to bottom of tubes *G*, *G′*, and *G″*. Regulate flow of gas thru reaction mixts in *G*, *G′*, and *G″* by stopcocks shown in top view, continuing passage of gas 2 hr. (At first rather slow stream of gas passes in, but during last 15 min., flow is fairly rapid.)

After reaction period discontinue flow of gas, and disconnect long tubes *F*, *F′*, and *F″* and outlet tubes of test tubes *G*, *G′*, and *G″* from *O* and *P*. Pull tubes *F*, *F′*, and *F″* just above surface of reaction mixt., and close with short pieces of rubber tubing having one end plugged with short piece of glass rod. Similarly close off outlet tubes, *N*, *N′* and *N″*. Place tubes contg reaction mixt. in cold room or icebox (temp. 8–10°) for 24 hr.

Transfer contents of *G*, *G′*, and *G″* to 1 L erlenmeyers, taking care to remove any material adhering either on inside or outside of tubes *F*, *F′*, and *F″*. Dil. reaction mixts to 500 ml with H_2O. Connect flasks to reflux condensers and boil 1 hr. Prep. 3 gooches in usual manner, dry at 105°, and weigh. Ignite one of weighed crucibles, *A*, on Bunsen burner, cool in desiccator, and reweigh. Let contents of flasks cool to room temp. and filter thru weighed gooches. Wash ppts collected in gooches with hot H_2O, dry at 105°, and weigh in weighing bottles. Ignite crude lignin in crucible *A* over Bunsen flame and det. wt ash. Place one of other 2 gooches in wide-neck Kjeldahl flask and det. % N in crude lignin as in **2.036**. If methoxyl in the lignin is to be detd, collect ppt from one of flasks in dried (105°) fritted glass crucible and proceed as in **38.029**.

Wt lignin = wt crude lignin − wt ash − wt crude protein ($N \times 6.25$). Calc. % lignin in original dry unextd material.

6.094 *Indirect Method (32)—First Action*

Ext. 1 g sample with alcohol-benzene (1+2) 4 hr in Soxhlet or comparable app. (extn vessel may be either coarse porosity Alundum or paper thimble, closed at top with filter paper or plug of cotton). Wash sample in thimble with suction, using 2 small portions alcohol followed by 2 small portions ether. Heat at 45° in non-sparking oven to drive off ether, and transfer sample to 250 ml wide-mouth erlenmeyer. Add 40 ml *1% soln of pepsin in 0.1N HCl*, wetting sample well by adding small portion of the soln, stirring or shaking thoroly, and finally washing down sides of flasks with remainder of soln. Incubate at 40° overnight.

Add 20–30 ml hot H_2O and filter, using filter stick. (Filter sticks are made with Pyrex fritted glass disk, 30 mm diam., medium porosity. Thin layer of pre-ashed diatomaceous earth (Hyflo Supercel, or similar filter-aid) is sucked onto disk from H_2O suspension. This is usually sufficient for easy filtration; if not, add extra Supercel to material being filtered. Some sticks filter slowly with some samples. It is advisable to obtain more than needed and discard slow-filtering ones. It is convenient to arrange filter sticks in set of 12 attached to vac. manifold by rubber tubing.)

Repeat washing twice and then wash residue into flask by forcing 7–8 ml 5% H_2SO_4 downward thru filter stick, using air pressure. Wash stick further with the H_2SO_4, finally adding enough to bring total vol. to ca 150 ml. Reflux vigorously on hot plate 1 hr, adding H_2O occasionally to maintain original vol. Filter off acid. Wash residue with three 20–30 ml portions hot H_2O, two 15–20 ml portions alcohol, and two 15 ml portions ether. Leave vac. on few min. to dry residue, and transfer from stick to flask by tapping and brushing. Heat to drive off any residual ether. If disk formed upon drying is difficult to break up into finely divided state (sometimes in case of immature plant samples), disperse residue in ether in flask and then boil off ether on steam bath. Add 20 ml 72% H_2SO_4 at 20° to residue and hold 2 hr at 20°, stirring occasionally. Add 125 ml H_2O, filter, wash once with 20 ml hot H_2O, and filter again. Wash

residue from filter stick and reflux as before 2 hr, using 150 ml 3% H_2SO_4. Filter residue onto gooch with asbestos pad and wash with hot H_2O until free of acid. Dry at 105–110° and det. lignin by loss in wt on ignition at 600°.

PIGMENTS

Chlorophyll—Official

Photoelectric Colorimetric Method for Total Chlorophyll Only (33)

6.095 APPARATUS

(**a**) *Mortar and pestle.*—Deep glass mortar ca 4" i.d. with well-defined lip is recommended.

(**b**) *Photoelectric colorimeter.*—Calibrate for chlorophyll, using plant ext. as in **6.097** and light filters with max. transmission near 660 mμ. (Combination of Corning H. R. light filters Nos. 243 and 396 is suitable.)

(**c**) *Wash bottles.*—Type fitted with rubber bulb, permitting operation with one hand, is recommended.

(**d**) *Waring blendor or similar high speed blender.*—(Vessels similar to No. 3, shown in *J. Assoc. Offic. Agr. Chemists* **25**, 583(1942), possess advantages over original blender container.)

6.096 REAGENTS

(**a**) *Acetone.*—(1) Undild acetone and (2) 85% soln by vol. Commercial acetone, tech. grade, is satisfactory.

(**b**) *Quartz sand.*—Acid-washed and dried.

6.097 DETERMINATION

Select field material carefully to insure representative sample. Remove representative portion from field sample, and if fresh, cut finely with hand shears and mix as thoroly as possible. Grind dried material in mill and mix thoroly.

Weigh 1–5 g into mortar and add small quantity (ca 0.1 g) $CaCO_3$ or Na_2CO_3. Macerate tissue with pestle, add quartz sand, and grind short time; then add 85% acetone, little at time, and continue grinding until tissue is finely ground. Transfer mixt. to funnel, filter with suction, and wash residue with 85% acetone. Return residue to mortar with more 85% acetone and grind again. Filter and wash as before. Repeat procedure until tissue is devoid of green color and washings are colorless. (It is advisable to grind residue at least once with undild acetone and then to add enough H_2O at end to bring acetone concn to 85%. High speed blender may be used instead of the mortar to macerate and ext. the tissue (*see* **6.100**), but each investigator should satisfy himself that device used exts tissue completely.) When extn is complete, transfer filtered ext. to vol. flask of appropriate size and dil. to vol.

Measure transmittance of soln with photoelec. colorimeter, and read quantity of chlorophyll present from curve relating transmittance and concn. Express chlorophyll values as mg/g tissue, or in other convenient manner.

Calibrate photoelec. colorimeter as follows: Ext. sample of fresh, green leaf material with 85% acetone, filter, wash residue, and dil. ext. to vol. as above. Make series of dilns of ext. and measure transmittance of original and of each of dild solns with instrument in same manner as when chlorophyll prepn is being used as calibration std. Transfer aliquot of original ext. to ether and evaluate total chlorophyll spectrophotometrically as in **6.100**(**b**) and (**c**). From value thus obtained, calc. chlorophyll content of original ext. and that of each of dild solns, and construct curve relating concn of chlorophyll with transmittance or absorbance as usual.

Spectrophotometric Method for Total Chlorophyll and the a and b Components (34, 35)

6.098 APPARATUS

Use app. in **6.095** (except photoelec. colorimeter), plus following:

(**a**) *Scrubbing tubes for washing ether solns.*—Open tubes of ca 20 mm diam., to one end of each of which is sealed tube of smaller diam. drawn to fine jet at lower end.

(**b**) *Spectrophotometer.*—Capable of isolating spectral region of ca 3 mμ near 660 mμ with negligible stray radiation. Tubulated cells with tightly fitting glass stoppers are recommended for work with ether.

6.099 REAGENTS

Those listed in **6.096** and following:

Ether.—Commercial grade ether is satisfactory without further purification.

6.100 DETERMINATION

(Wash glassware with concd Na_3PO_4 soln to remove traces of acid that may decompose chlorophyll.)

(**a**) *Extraction of chlorophyll from tissue.*—Select and prep. sample as in **6.097.** Disintegrate weighed portion (2–10 g, depending on chlorophyll content) of fresh plant tissue in blender cup that contains small quantity (ca 0.1 g) $CaCO_3$, or by use of mortar as in **6.097.** After tissue is thoroly disintegrated, filter ext. thru büchner fitted with quant. paper. Wash residue with 85% acetone, **6.096(a)**, and if necessary, use little ether to remove last traces of pigment. If extn is incomplete, return residue and paper to blender container with more 85% acetone and repeat extn. Filter and wash, as directed previously, into flask contg first filtrate. Transfer filtrate to vol. flask of

appropriate size and dil. to vol. with 85% acetone.

Pipet aliquot of 25–50 ml into separator contg ca 50 ml ether. Add H_2O carefully until it is apparent that all fat-sol. pigments have entered ether layer. Drain and discard H_2O layer. Place separator contg ether soln in upper rack of support. Add ca 100 ml H_2O to second separator placed in rack below first. Set scrubbing tube in place and let ether soln run thru it to bottom of lower separator and rise in small droplets thru the H_2O. When all soln has left upper separator, rinse it and scrubbing tube with little ether added from medicine dropper. Place scrubbing tube in upper separator and exchange its place in the support with separator now contg ether soln. Drain and discard H_2O in upper separator, add similar portion of fresh H_2O to lower separator, and repeat washing process. Continue washing ether soln until all acetone is removed (5–10 washings). Then transfer ether soln to 100 ml vol. flask, dil. to vol., and mix.

(**b**) *Spectrophotometric measurements.*—Add ca teaspoonful anhyd. Na_2SO_4 to 60 ml reagent bottle, and fill it with ether soln of pigment. When this soln is optically clear, pipet aliquot into another dry bottle and dil. with enough dry ether to cause $\log_{10} I_0/I$ value to fall between 0.2 and 0.8 at wavelength to be used. (Most favorable value is near 0.6 at 660 mμ, since such soln yields satisfactory value at 642.5 mμ.)

Fill 2 clean g-s. absorption cells with dry ether from pipet and polish outside surfaces of each, first with cotton wet with alcohol and then with dry cotton. Place cells in instrument, and det. whether each gives same galvanometer deflection. If not, clean again or select cells that do, and do this daily. Empty one cell, fill it with the dried ether soln, and place in instrument. Adjust entrance and exit slits until spectral region isolated is 3–4 mμ at 660.0 mμ.

Det. whether instrument is in proper adjustment for wavelength by taking readings thru solvent and soln at intervals of 1 mμ from 658–665 mμ. Calc. $\log_{10} I_0/I$ value for each wavelength at which readings were taken. Highest value should be at 660.0 mμ; if not, adjust instrument until it is, or make the 660.0 mμ readings at wavelength setting that gave highest value. In case of grating instrument apply same correction at 642.5 mμ; however, with prism instrument, correction at 642.5 mμ must be obtained from wavelength calibration curve for particular instrument in use. Calibrate instrument for wavelength in this way often enough to insure that it remains in proper adjustment. Take I_0 and I readings at 660.0 and 642.5 mμ (or corrected settings) for each unknown soln.

(**c**) *Calculation of chlorophyll concentration.*—Calc. $\log_{10} I_0/I$ values for each of readings made, substitute them in following simplified equations, and solve for total chlorophyll and each of *a* and *b* components as follows:

$$(1)\quad \text{Total chlorophyll (mg/L)} = 7.12 \log_{10} \frac{I_0}{I} \text{ (at 660.0 m}\mu) + 16.8 \log_{10} \frac{I_0}{I} \text{ (at 642.5 m}\mu).$$

$$(2)\quad \text{Chlorophyll } a \text{ (mg/L)} = 9.93 \log_{10} \frac{I_0}{I} \text{ (at 660.0 m}\mu) - 0.777 \log_{10} \frac{I_0}{I} \text{ (at 642.5 m}\mu).$$

$$(3)\quad \text{Chlorophyll } b \text{ (mg/L)} = 17.6 \log_{10} \frac{I_0}{I} \text{ (at 642.5 m}\mu) - 2.81 \log_{10} \frac{I_0}{I} \text{ (at 660.0 m}\mu).$$

6.101 SUPPLEMENTARY INFORMATION

Factors involved in spectrophotometric analysis of the chlorophyll system have been discussed in detail by Comar and Zscheile (*35*). These authors used Beer's law in form:

$$c = \frac{\log_{10} I_0/I}{\alpha l},$$

where I_0 is intensity of light transmitted by solvent-filled cell; I is intensity of light transmitted by soln-filled cell; c is concn of chlorophyll (g/L); α is specific absorption coefficient; and l is thickness of soln layer in cm.

Since at given wavelength, observed $\log_{10} I_0/I$ value of soln having 2 components represents sum of $\log_{10} I_0/I$ values of each of components, following equation holds in case of chlorophylls *a* and *b* at given wavelength:

$$(4)\quad (\log_{10} I_0/I)_{\text{observed}} = (\log_{10} I_0/I)_a + (\log_{10} I_0/I)_b.$$

If 1 cm cell is used, this equation may be expressed as:

$$(5)\quad (\log_{10} I_0/I)_{\text{observed}} = \alpha_a c_a + \alpha_b c_b.$$

Concns of chlorophylls *a* and *b* in given ether soln can now be calcd by equation (5) as follows:

(**a**) Det. $\log_{10} I_0/I$ values for the soln at 2 different wavelengths (660.0 and 642.5 mμ have been found advantageous for this purpose).

(**b**) Select from table proper specific absorption coefficients corresponding to wavelengths used.

(**c**) Substitute observed $\log_{10} I_0/I$ value and

specific absorption coefficient in equation (5) for each of the 2 wavelengths used (as illustrated for 660.0 and 642.5 mμ in equations (6) and (7)). Solve these 2 equations simultaneously for 2 unknowns, the concns of chlorophylls *a* and *b*.

(6) $\log_{10} I_0/I$ (at 660.0 mμ) $= 102c_a + 4.50c_b$.

(7) $\log_{10} I_0/I$ (at 642.5 mμ) $= 16.3c_a + 57.5c_b$.

Equations (1), (2), and (3) were derived in this way.

Criterion for accuracy of chlorophyll values detd by spectrophotometric method is agreement between analytical results as detd from measurements at different wavelengths. It has been demonstrated by Comar and Zscheile (*35*) that measurements at 660.0 and 642.5 mμ are convenient for routine analysis; however, readings may be made at other wavelengths to check these values. Specific absorption coefficients for chlorophylls *a* and *b* in ether soln that may be used for this purpose are presented in following table:

Absorption constants used in analysis (after Comar and Zscheile (35))

WAVELENGTH mμ	SPECIFIC ABSORPTION COEFFICIENTS (FOR ETHER SOLNS) *Chlorophyll a*	*Chlorophyll b*
660.0	102	4.50
642.5	16.3	57.5
600.0	9.95	9.95
581.0	8.05	8.05
568.0	7.11	7.11
613.0	15.6	8.05
589.0	5.90	10.3

These values may be used for calcns as follows:

(a) Values for total chlorophyll and % composition may be calcd from absorption values at 660.0 and 642.5 mμ as described.

(b) Check values for total chlorophyll may be calcd from absorption values at intersection points 600.0, 581.0, and 568.0 mμ.

(c) Check values for % composition may be calcd from absorption values for each of points 613.0 and 589.0 mμ in combination with value of total concn obtained from (a) or (b).

6.102 Carotenes—*Official*—*See* **39.014–39.017**

TOBACCO

Nitrogen—First Action

Kjeldahl Method for Samples Containing Nitrates

(For nitrate-free samples omit salicylic acid and thiosulfate treatment.)

6.103 REAGENTS

See **2.034** and the following:

(a) *Sodium hydroxide-thiosulfate soln.*—Dissolve 500 g NaOH pellets and 40 g $Na_2S_2O_3.5H_2O$ in H_2O and dil. to 1 L.

(b) *Methyl red indicator.*—Dissolve 1 g in 200 ml alcohol; *or* prep. mixed indicator by dissolving 0.8 g Me red and 0.2 g methylene blue in 500 ml alcohol.

6.104 APPARATUS

See **2.035**.

6.105 DETERMINATION

Place weighed sample (1–2 g) in digestion flask. Add vol. H_2SO_4 (contg 2 g salicylic acid/40 ml) corresponding to wt sample (35 ml for 1 g, 40 ml for 2 g for NO_3-contg samples; 20 and 25 ml, resp., for NO_3-free samples). Shake until thoroly mixed and let stand 30 min. or more with occasional shaking; then add 5 g $Na_2S_2O_3.5H_2O$. Shake, let stand 5 min., and heat carefully until frothing ceases. Turn off heat, add 0.7 g HgO (or metallic Hg) and 15 g K_2SO_4, and boil briskly 1–1.5 hr after soln clears.

Cool, add ca 200 ml H_2O, cool to ca room temp., and add few Zn granules. Tilt flask and carefully add 50 ml NaOH-thiosulfate soln without agitation. Immediately connect flask to distn bulb on condenser whose tip is immersed in 50 ml std 0.1*N* acid in receiving flask. Then rotate digestion flask carefully to mix contents. Heat until at least 150 ml distillate collects, and titr. excess acid with std base, using Me red or mixed indicator. Correct for blank detn on reagents.

Total Alkaloids (As Nicotine)

Distillation Method (36)—First Action

6.106 APPARATUS

(a) *Distillation apparatus.*—App. of **4.102**, Griffith (*37*) (available from Consolidated Glass Products Co., Kingsport, Tenn.), or other suitable steam distn app. may be used.

(b) *Spectrophotometer.*—Beckman Model DU or other instrument capable of accurately measuring absorbance in 200–300 mμ range, equipped with 1 cm quartz cells.

6.107 REAGENTS

(a) *Alkali-salt soln.*—Dissolve 300 g NaOH in 700 ml H_2O and sat. with NaCl.

(b) *Silicotungstic acid soln (for gravimetric determination).*—Dissolve 120 g $SiO_2.12WO_3.26H_2O$ in H_2O and dil. to 1 L. (Soln should be clear and free from green color.)

6.108 STANDARDIZATION

Purify best grade of nicotine commercially available by successive vacuum distns until center cuts from 2 successive distns have same absorptivity at 259 mμ (ca 34.3). Weigh accurately ca 0.2 g purified nicotine; dissolve in and dil. to 1 L with ca 0.05*N* HCl. Dil. 10 ml aliquot of this

soln to 100 ml with ca $0.05N$ HCl. Det. absorbance, A, at 259 mμ and calc. absorptivity $a = A/c \times b$, where c is concn of nicotine in g/L and b is cell length in cm.

6.109 DISTILLATION

Weigh accurately 2–5 g tobacco sample and transfer to distn flask or app. (If final detn of nicotine is gravimetric, use sample contg at least 0.1 g alkaloids; if spectrophotometric, use sample of at least 2 g.) (If Griffith still is used, use 0.05–0.2 g sample.) Place 25 ml HCl (1+4) in suitable receiver (1 L vol. flask is desirable) and place receiver so that condenser tube dips into HCl soln. (With Griffith still, use 10 ml HCl (1+4) in 250 ml vol. flask.) Add 50 ml alkali-salt soln to distn flask so that sample is rinsed into bottom of flask. (With Griffith still, use 5 ml alkali-salt soln.) If large vol. of liquid is required for proper function of still, add more alkali-salt soln; do not dil. Connect flask to app. immediately and steam distill with as rapid current of steam as be can condensed efficiently. Effluent condensate should not be above room temp. Apply heat to distn flask from burner, mantle, or other heat source to keep vol. in flask approx. constant. Collect ca 900 ml of condensate (or distill addnl 100 ml after condensate shows no nicotine by silicotungstic acid test). (With Griffith still, collect 225 ml.) Dil. distillate to vol.

6.110 DETERMINATION

(a) *Spectrophotometric.*—Dil. aliquots of distillate (if necessary) with $0.05N$ HCl so that absorbance at 259 mμ is 0.5–0.8 and read absorbance at 236, 259, and 282 mμ. Calc. corrected $A'_{259} = 1.059$ [observed $A_{259} - \frac{1}{2}(A_{236} + A_{282})$] after correcting all observed A values to original distillate vol. basis. Concn, c, of alkaloids as nicotine in g/L is given by $c = A'_{259}/a \times b$, where a is absorptivity at 259 mμ, and b is cell length in cm. Calc. % alkaloid (as nicotine) $= c \times$ vol. distillate (L) $\times$ 100/g sample.

(b) *Gravimetric.*—Det. alkaloids in distillate as in **4.102**, but double amount of silicotungstic acid specified, i.e., 2 ml/each 10 mg alkaloids expected.

Cundiff-Markunas Method—First Action

(Total alkaloids (as nicotine), tertiary alkaloids (as nicotine), and secondary alkaloids (as nornicotine).)

6.111 REAGENTS

(a) *Benzene-chloroform soln.*—Mix equal parts by vol. of benzene and $CHCl_3$ and sat. with H_2O.

(b) *Sodium hydroxide soln.*—36%. Dissolve 500 g NaOH in H_2O and dil. to 1 L.

(c) *Dilute acetic acid.*—5%. Dil. 50 ml HOAc to 1 L with H_2O.

(d) *Crystal violet indicator.*—Dissolve 0.5 g crystal violet in 100 ml HOAc.

(e) *Dilute perchloric acid.*—$0.025N$. Add 4.7 ml 72% $HClO_4$ to freshly opened 5 lb bottle HOAc and mix. Stdze as follows: Accurately weigh 0.1 g KH phthalate (NBS) into 125 ml erlenmeyer, add 50 ml HOAc, and heat to dissolve. Cool, add 2 drops crystal violet indicator, and titr. to blue-green end point. Perform blank titrn on 50 ml HOAc and 2 drops indicator soln, and correct vol. of titrant.

$$N = \frac{\text{wt KH phthalate} \times 4.897}{\text{ml } HClO_4}$$

6.112 DETERMINATION

Accurately weigh 2.5 g of finely ground tobacco into 250 ml erlenmeyer. Add 15 ml 5% HOAc and swirl until tobacco is thoroly wetted. Pipet 100 ml of benzene-$CHCl_3$ soln into flask, and then 10 ml 36% NaOH soln. Stopper flask tightly and shake 20 min., using wrist-action shaker. Add 4.5–5 g (2 teaspoonfuls) Filter-Cel, mix, and filter most of benzene layer thru Whatman No. 2 paper into second flask. If filtrate has any turbidity, add 2–2.5 g (1 teaspoonful) addnl Filter-Cel and refilter thru Whatman No. 2 paper. Filtrate must be clear.

Pipet 25 ml aliquots of filtrate into each of two 125 ml erlenmeyers. Pass stream of air over surface of soln in first flask 5 min., add 2 drops of crystal violet indicator, and titr. to green end point with $0.025N$ $HClO_4$. Add 1.0 ml Ac_2O to second flask and let stand at least 15 min. Add 25 ml HOAc and 2 drops of crystal violet indicator and titr. to blue-green end point with $0.025N$ $HClO_4$. Take first appearance of blue-green color thruout soln as end point. For each series of analyses perform blank titrns and correct respective vols of titrant.

Calculate percentage of alkaloids as follows: % total alkaloids (as nicotine) $= V_1 \times N \times 32.45$/wt sample; % tertiary alkaloids (as nicotine) $= (2V_2 - V_1) \times N \times 32.45$/wt sample; % secondary alkaloids (as nornicotine) $= 2(V_1 - V_2) \times N \times 29.64$/wt sample; where V_1 = vol. titrant for non-acetylated aliquot; V_2 = vol. titrant for acetylated aliquot; and N = normality $HClO_4$.

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7. Baking Powders and Baking Chemicals

7.001 Preparation of Sample—Official

Remove entire sample from package, pass thru No. 20 sieve, and mix thoroly.

Total Carbon Dioxide (*1*)—Official

(Applicable to baking powders contg added $CaCO_3$)

7.002 REAGENT

Displacement soln.—Dissolve 100 g NaCl or $Na_2SO_4.10H_2O$ in 350 ml H_2O. Add ca 1 g $NaHCO_3$ and 2 ml Me orange, **3.001(e)**, and then enough H_2SO_4 (1+5) or HCl (1+2) to make just acid (decided pink). Stir until all CO_2 is removed. This soln is used in gas-measuring tube and leveling bulb and seldom needs replacement.

7.003 APPARATUS

Chittick apparatus.—Fig. 17. Connect decomposition flask, *A*, by glass T-tube, *B*, provided with stopcock, *C*, to graduated gas-measuring tube, *D*, connected in turn with leveling bulb, *E*. For *A* always use 250 ml wide-mouth extn flask of Pyrex or other resistant glass fitted with 2 hole rubber stopper, thru one hole of which passes extended tip of 25 ml buret, *F*, and thru other, glass tube of same diam. as connecting T-tube. Use buret graduated in ml at 20°, numbered at 5 ml intervals, and fitted with extra-long tip bent to pass thru rubber stopper. Connect glass tube leading from decomposition flask to T-tube with rubber tubing to permit rotation of flask. Use gas-measuring tube graduated in ml at 20° with zero mark at point 25 ml below top marking to allow for graduating upward from 0 to 25 ml and downward from 0 to 200 ml. Connect gas-measuring tube to ca 300 ml leveling bulb with long rubber tube.

(Available from E. H. Sargent & Co., 4647 West Foster Ave., Chicago, 30 Ill.)

7.004 DETERMINATION (*2*)

Weigh 1.7 g prepd sample, **7.001**, into flask *A* and connect flask with app., Fig. 17. Open stopcock *C*, and using leveling bulb *E*, bring displacement soln to 10 ml graduation above zero mark. (This 10 ml is practically equal in vol. to that of acid to be used in decomposition.) Let app. stand 1–2 min. for temp. and pressure within app. to come to room conditions.

Close stopcock, lower leveling bulb somewhat to reduce pressure within app., and slowly add to decomposition flask from buret *F* 10 ml H_2SO_4 (1+5) or HCl (1+2). To prevent escape of liberated CO_2 thru acid buret into air, at all times during decomposition keep displacement soln in leveling bulb at level lower than that in gas-measuring tube. Rotate and then vigorously agitate decomposition flask to mix contents intimately. Let stand 5 min. to secure equilibrium. Equalize pressure in measuring tube, using leveling bulb, and read vol. of gas in tube. Observe temp. of air surrounding app. and also barometric pressure, and multiply ml gas evolved by factor given in table **43.027** for this temp. and pressure. Corrected reading/10 = % CO_2 by wt.

7.005 Residual Carbon Dioxide (*3*)—Official

(**a**) *After drying on water bath.*—Place 1.7 g baking powder in clean, dry, 250 ml wide-mouth Soxhlet extn flask *A*, **7.003.** Add 20 ml H_2O. Put flask *on cover* of H_2O bath (single or multiple) in which boiling H_2O is kept at constant level of 2″ below top of bath. (H_2O in bath must boil vigorously all thru detn. Opening in cover of bath must be 3″ diam. to prevent flask from touching H_2O.) Evap. contents of flask until no moisture is visible in residue or inside surface of flask. (Sample should be completely dry in 1.5–2 hr.) Leave flask on H_2O bath 2 hr more. Add 10 ml H_2O, and let stand until flask is at room temp. (ca 1 hr).

Det. CO_2 with Chittick app. as in **7.004,** using correction factors in **43.027.** Shake flask vigorously until further shaking produces no increase in reading.

(**b**) *After drying in oven.*—Place 1.7 g sample in clean, dry 250 ml wide mouth Soxhlet extn flask *A*, **7.003.** Tap flask to spread sample evenly on bottom. Add 10 ml H_2O with pipet. Stir with glass rod to break up powder that may have caked on bottom of flask. Wash down stirring rod and sides of flask with 10 ml H_2O. Place flask on shelf near center of air oven set at 100 ± 2°, and evap. to dryness. After 5 hr, remove from oven, add 10 ml H_2O, and cool to same temp. as air surrounding Chittick app., **7.003.** Det. CO_2 present in residue with Chittick app., using correction factors in **43.027.** Shake flask vigorously until further shaking produces no increase in reading.

7.006 Available Carbon Dioxide—Official

(Applicable to baking powders contg added $CaCO_3$)

Subtract the residual CO_2, **7.005,** from the total CO_2, **7.004.**

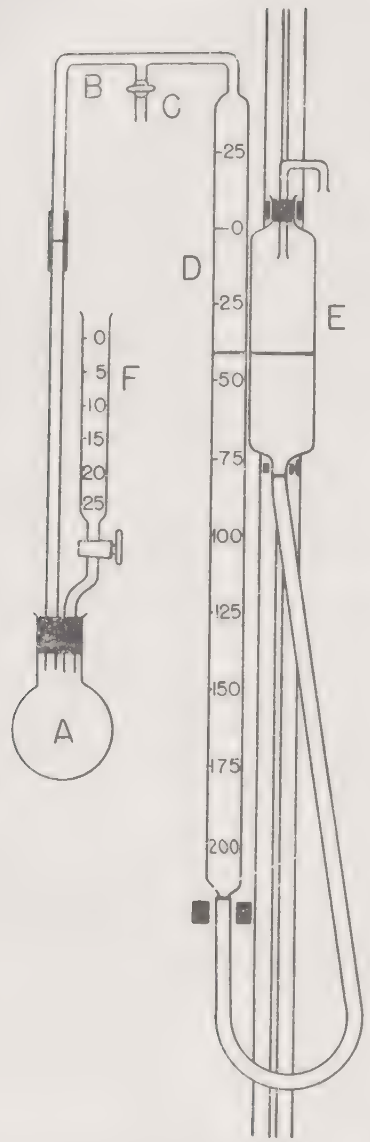

FIG. 17. CHITTICK APPARATUS FOR GASOMETRIC DETERMINATION OF CARBON DIOXIDE

Neutralizing Value

7.007 *Of Acid-Reacting Materials Other Than Phosphates—Official*

Dissolve 1 g sample in hot H_2O and titr. with $0.2N$ NaOH, using phthln. Express result as parts $NaHCO_3$ equiv. to 100 parts of the acid-reacting material.

7.008 *Of Monocalcium Phosphate (4)—Official*

Weigh 0.84 g sample into 375 ml casserole. Add 24 ml cold H_2O and, after stirring for moment, add exactly 90 ml $0.1N$ NaOH. Bring suspension to boil in exactly 2 min., and boil 1 min. While soln is still boiling hot, add 1 drop phthln, and back-titr. with $0.2N$ HCl until all pink disappears. Boil soln 1 min., and again add $0.2N$ HCl until pink just disappears. $90 - (\text{ml } 0.2N \text{ HCl} \times 2)$ = neutralizing value, parts $NaHCO_3$ equiv. to 100 parts of the phosphate.

7.009 *Of Anhydrous Monocalcium Phosphate (4)—Official*

Use 100 ml $0.1N$ NaOH and stir intermittently 5 min. before bringing to boil. Proceed as in **7.008.**

7.010 *Of Sodium Acid Pyrophosphate (4)—Official*

Weigh 0.84 g sample and 20 g NaCl into 375 ml casserole, and slowly add 25 ml H_2O while stirring. Stir and crush with flat-end rod 3–5 min. Add 90 ml $0.1N$ NaOH and 1 drop phthln, and titr. with $0.2N$ HCl until pink disappears. If "starch filled" or 50% neutralizing strength pyrophosphate is being titrd, use 70 ml of the NaOH. Ml $0.1N$ NaOH $-$ (ml $0.2N$ HCl $\times 2$) = neutralizing value, parts $NaHCO_3$ equiv. to 100 parts Na acid pyrophosphate.

7.011 Tartaric Acid, Free or Combined (Qualitative Test) *(5)*—**First Action**

(Applicable in presence of phosphates)

Shake ca 5 g sample repeatedly with ca 250 ml cold H_2O in flask, and let insol. portion settle. Decant soln thru filter, and evap. filtrate to dryness. Powder residue, add few drops 1% resorcinol soln, **29.121,** and ca 3 ml H_2SO_4, and heat slowly. Tartaric acid is indicated by rose-red color, discharged on diln with H_2O.

Cream of Tartar and Free Tartaric Acid in Tartrate Powders *(6)*

Total, Combined, and Free Tartaric Acid—Official

7.012 DETERMINATION

To 2.5 g sample in 250 ml vol. flask add 100 ml H_2O at ca 50°, and hold at room temp. ca 30 min., shaking occasionally. Cool, dil. to mark with H_2O, shake vigorously, and filter thru large fluted paper. Pipet 2 portions of 100 ml each of *clear* filtrate into 250 ml beakers, and evap. to ca 20 ml. To one portion add 3.5 ml ca $1N$ KOH. Mix well, and add 2 ml HOAc. Again mix well and add 100 ml alcohol, stirring constantly. Treat other portion similarly, but use $1N$ NaOH instead of KOH. Then treat each mixt. separately as follows: Cool to ca 15°, stir vigorously ca 1 min., and leave in refrigerator overnight. Collect ppt in gooch on thin, tightly tamped pad of asbestos. Rinse beaker with ca 75 ml ice-cold 80% alcohol, carefully washing down sides of beaker. Finally wash sides of crucible with 25 ml alcohol and suck dry. Transfer contents of crucible to original beaker with ca 100 ml hot H_2O, and titr. with

0.1N alkali, using phthln. Designate titer of portion treated with KOH as "A" and that treated with NaOH as "B."

7.013 CALCULATIONS

% total tartaric acid = 1.5(A + 0.6).
% cream of tartar = 1.88(B + 0.6).
% free tartaric acid = 1.5(A − B).
In above formulas "0.6" represents solubility of cream of tartar in reaction mixt. in terms of 0.1N alkali.

Free Tartaric Acid (Direct Determination)—Official

7.014 REAGENT

Saturated alcohol.—To ca 50 g finely powd. pure cream of tartar in erlenmeyer add ca 100 ml alcohol and 100 ml H_2O, shake vigorously several min., and let stand 15 min., shaking occasionally. Filter on paper in büchner; wash with ca 200 ml alcohol (1 + 1), then with alcohol, and finally with ether. Dry at temp. of boiling H_2O. To 500 ml *absolute* alcohol add ca 5 g of the purified cream of tartar and let stand 2 hr, shaking occasionally. Properly purified cream of tartar requires not > 0.15 ml 0.1N alkali to neutralize 100 ml of mixt. of 50 ml $CHCl_3$ and 150 ml of the satd alcohol.

7.015 DETERMINATION

Weigh 1.25 g sample into *absolutely dry* 200 ml vol. flask, add 50 ml $CHCl_3$, and let stand ca 5 min., shaking occasionally. (Discard detn if upon addn of $CHCl_3$, powder sticks to bottom of flask, indicating moisture.) Add 100 ml of the satd alcohol, shake ca 5 min., and let stand 30 min., shaking at frequent intervals. (It is not necessary to filter the alcohol reagent.) Dil. to mark with the satd alcohol, shake few min., and filter thru large fluted paper. Titr. 100 ml clear filtrate with 0.1N alkali, using phthln. Ml alkali used × 1.2 = % free tartaric acid.

7.016 *Free Tartaric Acid (Qualitative Test)—Official*

Ext. 5 g sample with absolute alcohol and evap. alcohol from ext. Dissolve residue in NH_4OH (1 + 10), transfer to test tube, add good-size crystal of $AgNO_3$, and heat gently. Tartaric acid is indicated by formation of Ag mirror. (If desired, alc. ext. may be tested as in **7.011**.

Starch

7.017 *Direct Inversion Method—Official*

(For baking powders and baking chemicals free from Ca)

Weigh 5 g sample into 500 ml vol. flask and proceed as in **22.043**.

7.018 *Indirect Method (7)—Official*

(For baking powders and baking chemicals contg Ca)

Mix 5 g sample with 200 ml HCl (1 + 11) in 500 ml vol. flask and let mixt. stand 1 hr, shaking frequently. Filter on 11 cm hardened paper, taking care to obtain clear filtrate. Rinse flask once without attempting to remove all starch, and wash paper twice with cold H_2O. Carefully wash starch from paper back into flask with 200 ml H_2O. Add 20 ml HCl (sp. gr. 1.125) and proceed as in **22.043.** (Treatment with the HCl, without dissolving starch, effectively removes Ca, which otherwise would be pptd as tartrate by the alk. Cu soln.)

Aluminum

Qualitative Test (8)—Official

(In presence of phosphates)

7.019 REAGENTS

(**a**) *Ammonium acetate soln.*—50%. Dissolve 50 g NH_4OAc in 50 ml H_2O.

(**b**) *Aurintricarboxylic acid soln.*—0.1%. Dissolve 0.1 g aurintricarboxylic acid in H_2O and dil. to 100 ml.

7.020 DETECTION

Place 1 g sample in 250 ml beaker, add 5 ml ca 1N HCl and 20 ml H_2O, and heat until starch hydrolyzes. Add 100 ml cold H_2O, 5 ml *10% $NaNH_4HPO_4.4H_2O$ soln*, and 3 drops Me orange. Add NH_4OH dropwise until ppt forms or color changes; then add the 1N HCl dropwise until ppt dissolves or color changes. Add 2 or 3 drops excess of the 1N HCl. Add 5 ml of the aurintricarboxylic acid soln and let stand 1 min. Add the 50% NH_4OAc soln dropwise until ppt forms or color changes and then add 1 ml excess. Let stand 5 min., stirring occasionally, and filter portion of soln. Bright red ppt on filter paper indicates presence of Al.

7.021 **Insoluble Ash and Preparation of Solution** (*9*)—**Official**

Char 5 g sample in Pt dish at heat below redness (ca 500°). Boil carbonaceous mass with HCl (1 + 2.5), filter into 500 ml vol. flask, and wash with hot H_2O. Return residue, together with paper, to Pt dish, and burn to white ash. Boil again with the dil. HCl, filter, wash, unite the 2 filtrates, and dil. to 500 ml. Incinerate residue after last filtration and weigh ash insol. in acid.

7.022 **Iron and Aluminum** (*9*)—**Official**

Draw 100 ml aliquot prepd soln, **7.021,** and sep. SiO_2 if necessary. Mix soln with excess *10% $Na_2HPO_4.12H_2O$ soln*. Add NH_4OH until perma-

nent ppt is obtained, then HCl dropwise until ppt dissolves. Bring soln to boil and boil 2–3 min.; mix with considerable excess 50% NH_4OAc soln, **7.019**(**a**), and 4 ml HOAc (4+1). As soon as ppt of $AlPO_4$, mixed with $FePO_4$, settles, collect on filter, wash with hot H_2O, ignite, and weigh. Fuse mixed phosphates with 10 parts Na_2CO_3, dissolve in H_2SO_4 (1+6), reduce with Zn, and det. Fe by titrn with std $KMnO_4$ soln (1 ml = 1 mg Fe). Det. P_2O_5 in aliquot from **7.021** as in **2.019** or **2.022.** Wt mixed phosphates − wt ($Fe_2O_3+P_2O_5$) = wt Al_2O_3.

7.023 Calcium (*9*)—Official

Heat combined filtrate and washings obtained in **7.022** to 50°, and add excess satd NH_4 oxalate soln. Let stand in warm place until ppt settles, filter, wash ppt with hot H_2O, dry, and ignite over Bunsen burner and finally over blast lamp. Cool in desiccator and weigh as CaO.

7.024 Potassium and Sodium (*9*)—Official

Evap. aliquot prepd soln, **7.021,** nearly to dryness to remove excess HCl, dil., and heat to boiling. While soln is still boiling add 10% $BaCl_2.2H_2O$ soln as long as ppt forms, and then enough satd $Ba(OH)_2$ soln to make liquid strongly alk. After ppt settles, filter, and wash with hot H_2O; heat filtrate to boiling, add enough $(NH_4)_2CO_3$ soln (1 part $(NH_4)_2CO_3$ in 5 parts NH_4OH soln (1+12)) to ppt all the Ba, filter, and wash with hot H_2O. Evap. filtrate to dryness and ignite residue below redness to remove NH_4 salts. Add little H_2O and few drops of the $(NH_4)_2CO_3$ soln to residue. Filter into weighed Pt dish, evap., ignite below redness, and weigh mixed K and Na chlorides.

Digest residue with hot H_2O, filter thru small filter, and dil. filtrate, if necessary, to provide at least 20 ml of liquid for each 100 mg K_2O. Acidify with few drops HCl and add excess Pt soln, **2.059**(**b**). Evap. on H_2O bath to thick paste; treat residue repeatedly with 80% alcohol, decanting thru weighed gooch or other filter; transfer ppt to filter, and wash thoroly with the 80% alcohol. Dry 30 min. at 100° and weigh. Calc. K found to its equiv. of KCl and subtract result from wt mixed chlorides to obtain wt NaCl.

7.025 Phosphorus—Official

Mix 5 g sample with little $Mg(NO_3)_2$ soln, **2.017**(**e**), dry, ignite, dissolve in HCl (1+2.5), and dil. to definite vol. In aliquot of soln det. P_2O_5 as in **2.019** or **2.022.**

7.026 *Qualitative Test—Official*

Add 10 ml H_2O to 1–2 g sample in 150 ml beaker. Make just acid with HNO_3, filter, take equal vols filtrate and NH_4 molybdate soln, **2.017**(**a**), and warm at 40–50°. Yellow ppt indicates presence of phosphate.

7.027 Sulfate (*10*)—Official

Boil 5 g sample 1.5 hr with mixt. of 300 ml H_2O and 15 ml HCl. Filter, wash filter thoroly with hot H_2O, cool combined filtrate and washings, and dil. to 500 ml with H_2O. Det. sulfate in 100 ml aliquot as in **6.058.**

7.028 Ammonia—Official

To 2 g sample in distn flask add 300–400 ml H_2O and excess of NaOH soln (1+1), connect with condenser, and distill into measured vol. std acid. Titr. excess acid in distillate with std alkali, using Me red.

7.029 Arsenic—Official

Place 5 g sample directly in generator, **24.002**(**a**); add 10 ml H_2O, little at time to prevent foaming over, and then 15 ml As-free HCl, adding it dropwise until foaming ceases. Heat on steam bath until drop of mixt., when dild and treated with I soln, shows no blue color. Then dil. to ca 30 ml with H_2O and continue as in **24.005,** beginning "add 5 ml of the KI reagent . . ." Prep. blank and stds for comparison, using As-free HCl of same concn as that used in detn.

7.030 Fluorine—Official—*See* 24.025–24.031

7.031 Lead—Official—*See* 24.037–24.051

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(*6*) J. Assoc. Offic. Agr. Chemists **13,** 385(1930); **22,** 599(1939).
(*7*) Conn. Agr. Expt. Sta. Rpt. 1900 (II), p. 174.
(*8*) J. Am. Chem. Soc. **47,** 142(1925); J. Assoc. Offic. Agr. Chemists **34,** 61(1951); **35,** 57 (1952).
(*9*) Conn. Agr. Expt. Sta. Rpt. 1900, p. 178.
(*10*) U. S. Dept. Agr. Bur. Chem. Bull. **13** (V), p. 596; Conn. Agr. Exp. Sta. Rpt. 1900, p. 179.

8. Beverages: Non-Alcoholic and Concentrates

8.001 Preliminary Examination—Procedure

Note and record (*a*) appearance, whether bright or turbid, or any sediment; (*b*) color and depth of color; (*c*) odor, whether fruity, foreign, or artificial; (*d*) taste, whether tart or sweet, fruity, artificial, or foreign, and whether any synthetic substance can be identified by odor or taste.

8.002 Specific Gravity—Official—*See* 9.011

8.003 Alcohol—Official—*See* 9.013, 9.016, and 9.021

8.004 Total Solids—Official—*See* 29.007 or 29.008

Sucrose

8.005 *By Polarization—Official*

Det. by polarizing before and after inversion as in **29.025** or **29.026.**

8.006 *By Reducing Sugars Before and After Inversion—Official—See* **29.032**

8.007 Reducing Sugars—Official

Use value obtained for reducing sugars before inversion, **8.006.**

8.008 Commercial Glucose—Procedure—*See* 29.034

8.009 Ash—Official

Proceed as in **29.012** or **29.013,** using sample contg not >10 g solids.

8.010 Soluble and Insoluble Ash—Official

Proceed as in **29.015,** using ash of **8.009.**

8.011 Alkalinity of Soluble Ash—Official

Proceed as in **29.016,** using sol. ash of **8.010.**

8.012 Alkalinity of Insoluble Ash—Official

Proceed as in **29.017,** using insol. ash of **8.010.**

8.013 Analysis of the Ash—Official—*See* Chap. 20

8.014 Monochloroacetic Acid—Official *See* 27.037(b), 27.038(b), and 27.041

8.015 Quaternary Ammonium Compounds—Official—*See* 27.060–27.061

8.016 Total Acidity—Official *See* 9.070

8.017 Preparation of Sample for Determination of Dibasic Acids—First Action

(**a**) *Alcoholic products.—See* **9.071.**

(**b**) *Non-alcoholic products.*—Use sample contg not >30 g solids and not >200 mg acid to be detd, as calcd from acidity. Evap. to 30 ml if necessary, and treat as in **8.018–8.021.**

8.018 Tartaric Acid—First Action

Designate as *A* the ml of 1*N* alkali required to neutralize sample, add 3 ml 1*N* H_2SO_4, heat to 50°, and continue as in **20.044,** beginning "Transfer adjusted sample into 250 ml vol. flask . . . "

8.019 Citric Acid—First Action

Transfer sample to 250 ml vol. flask, using enough H_2O to make total vol. of 70 ml, and continue as in **20.047,** beginning "Add 2 ml 1*N* HNO_3 . . . "

8.020 Total Malic Acid (Laevo and Inactive)—First Action

Proceed as in **8.018** to obtain filtrate and washings from KH tartrate; then evap. soln to ca 15 ml and continue as in **20.053,** beginning "Transfer with small amount of H_2O . . . "

8.021 Laevo Malic Acid—First Action

Proceed as in **8.018** to obtain filtrate and washings from KH tartrate; proceed as in **20.061.**

8.022 Volatile Acids—Official—*See* 11.031

8.023 Esters—Official

Proceed as in **9.076,** collecting ca 300 ml distillate.

Methyl Anthranilate—Official

Colorimetric Method (1)

(Applicable to samples contg <500 mg/L)

8.024 REAGENTS

(**a**) *Dilute hydrochloric acid.*—Dil. 83 ml HCl to 1 L with H_2O.

(**b**) *Sodium nitrite soln.* Dissolve 2 g $NaNO_2$ in 100 ml H_2O.

(c) *Hydrazine sulfate soln.*—Dissolve ca 3 g $N_2H_4.H_2SO_4$ in 100 ml H_2O.

(d) *Sodium-α-naphthol-2-sulfonate soln.*—Dissolve 5 g of the sulfonate in 100 ml H_2O.

(e) *Sodium carbonate soln.*—Dissolve 25 g Na_2CO_3 in 75 ml H_2O.

(f) *Methyl anthranilate std soln.*—Dissolve 0.25 g Me anthranilate in 60 ml alcohol and dil. to 250 ml with H_2O.

8.025 APPARATUS

(a) *Steam generator filled with H_2O.*—Oil can holding 1 gal. serves purpose.

(b) *Distillation flask.*—Kjeldahl flask, ca 750 ml capacity, with shortened neck, ca 10″ over-all height.

(c) *Spray tube.*—Glass tube with small perforated bulb at end, passing thru rubber stopper and reaching to bottom of distn flask.

(d) *Connecting bulb.*—Kjeldahl bulb with bent connecting tube.

(e) *Worm condenser.*—With H_2O jacket 10–12″ long, and outlet tube reaching bottom of 500 ml erlenmeyer receiving flask.

8.026 DETERMINATION

Place just enough H_2O in receiving flask to cover or seal end of extended condenser tube. Place 10–100 ml sample in distn flask and add, if necessary, enough H_2O to make 100 ml. Insert stopper carrying spray tube and connecting bulb, and connect with condenser and receiving flask. Immerse distn flask in H_2O bath to level of contents. When sample reaches temp. of nearly boiling bath, connect with steam generator and pass steam rapidly thru sample until ca 300 ml distillate collects.

Disconnect app. and wash out condenser with little H_2O. Add to distillate 25 ml of the dil. HCl and 2 ml of the $NaNO_2$ soln, mix well, and let stand exactly 2 min. Add 6 ml of the $N_2H_4.H_2SO_4$ soln and mix well 1 min., so that liquid comes in contact with all parts of flask that soln may have touched when it contained free HNO_2. Keep liquid in flask in rapid motion, quickly add 5 ml of the Na α-naphthol-2-sulfonate soln, and then immediately add 15 ml of the Na_2CO_3 soln. Dil. colored soln to 500 ml with H_2O, mix, and compare color of aliquot with color of std, or set of stds, prepd as nearly as possible at same time. Calc. results as mg Me anthranilate/L.

Gravimetric Method (2)

(Applicable to samples contg 500 mg or more/L)

8.027 REAGENTS AND APPARATUS

(a) *α-Naphthol soln.*—Dissolve 0.2 g α-naphthol in 100 ml 30% alcohol.

(b) *Sodium bicarbonate soln.*—Dissolve 8.4 g $NaHCO_3$ in 100 ml H_2O.

(*See* **8.024** for other reagents and **8.025** for app.)

8.028 DETERMINATION

Place sample contg 50–125 mg Me anthranilate in distn flask and dil., if necessary, to 100 ml with H_2O. Steam distill as in **8.026**, collecting ca 400 ml distillate. Have H_2O in bath near b.p. when bath is placed under distn flask; also have H_2O in steam generator boiling, and connect immediately.

Wash out condenser with little H_2O and dil. distillate to 500 ml. Mix, and to 200 ml aliquot add 5 ml of the dil. HCl, **8.024(a)**, and 5 ml of the $NaNO_2$ soln, **8.024(b)**. Mix well and let stand 1 min. Mix 25 ml of the α-naphthol soln and 6 ml of the $NaHCO_3$ soln, pour diazotized soln into mixt., and let stand 10 min. Fold 2 Whatman 1 or S&S 595 papers, 12.5 cm diam., and det. difference in their wts by placing one on each pan of balance and counterpoising with added wts. Place heavier inside lighter paper, fit into funnel, and moisten. Pour mixt. thru this filter and wash ppt 7 or 8 times, using total of ca 100 ml H_2O. Fill filter only to ca 1 cm from top. Place funnel carrying filter and washed ppt in oven, and dry ca 10 min. at 100°. Sep. and dry filter papers ca 1 hr at same temp. Det. difference in wts, dry again, weigh again, and repeat procedure until difference in wts remains constant. (Constant difference in wts − original difference in wts of 2 papers) $\times 0.4935 =$ wt anthranilic acid ester, as Me anthranilate. Report as g/L.

8.029 Benzaldehyde (*3*)—**Official**

Measure 500 ml beverage, 100 ml flavoring sirup, or 10–25 ml flavor into distg flask. Add 32 ml alcohol, and in case of sirup or flavor, ca 300 ml H_2O, and proceed as in **9.080**.

8.030 Gamma Undecalactone (*4*)—**Official**

Proceed as in **9.077**, using 500 ml beverage, 100 ml flavoring sirup, or 10–50 ml flavor.

8.031 Essential Oils—First Action— *See* **19.103**

Caffeine (*5*)—**First Action**

8.032 APPARATUS

(a) *Continuous extractor.*—Similar to Fig. 63C, page 475; outer part of 43 mm o.d. tubing, 45 cm long, with side tube 25 cm above bottom, fitted with drip tip ₸ 24/40 joint; inner tube of 30 mm o.d. tubing, 39–40 cm long; receiver is 250 ml erlenmeyer with ₸ 24/40 joint.

(b) *Filtering device.*—Glass büchner with 30 mm fine fritted disk and 45 mm high side wall.

fitted with 2 interchangeable rubber stoppers, one to fit suction flask, other to fit 20×150 mm side arm test tube.

8.033 REAGENTS

(**a**) *Phosphomolybdic acid soln.*—Dissolve 10 g phosphomolybdic acid in ca 25 ml warm H_2O, cool to room temp., and dil. to 50 ml with H_2O. Let stand overnight and filter thru S&S 589 blue ribbon paper. Store in dark.

(**b**) *Caffeine std soln.*—1 mg/ml. Weigh 100 mg caffeine alkaloid, dissolve in H_2O, and dil. to 100 ml.

8.034 EXTRACTION

Place few glass beads in receiver, assemble extractor, and add 210–220 ml $CHCl_3$ to inner tube. Measure 150 ml sample in graduated cylinder and make alk. with ca 2 ml 10*N* NaOH, using litmus paper as indicator. Place funnel in top of extractor and add sample to inner tube, letting $CHCl_3$ in outer tube overflow into receiver. Remove funnel and attach condenser. If tip of condenser is more than 2 cm above inner tube, place small funnel with 2–3 cm stem in top of inner tube to prevent splashing. Ext. 2 hr, keeping steady stream of solvent flowing from condenser.

Remove hot plate and let receiver cool somewhat. Disconnect condenser and tilt extractor to permit as much $CHCl_3$ as possible to flow into receiver without letting any aq. phase rise into space between the 2 parts of extractor. Transfer entire contents of extractor to separator. Rinse extractor with H_2O and discard. Attach outer part of extractor to receiver and to condenser, and heat carefully until vol. $CHCl_3$ in receiver is 30–40 ml. Do not let soln bump or foam into extractor. Cool somewhat, drain $CHCl_3$ ext. from separator into receiver, and distill the $CHCl_3$ into extractor as before until 10–15 ml remains in receiver. Cool $CHCl_3$ in receiver and transfer to tared 50 ml beaker, rinsing with several small portions $CHCl_3$. Evap. to dryness and weigh.

If residue wt is 5 mg or less, dissolve in 5 ml H_2O, filter thru very small circle of paper into 50 ml beaker, and wash beaker and paper with 5 ml H_2O; if wt is >5 mg, dissolve, using several successive 5 ml portions H_2O, and filter into vol. flask (25 ml for residue of 15 mg or less; 50 ml if >15 mg). Dil. to mark with H_2O and mix.

8.035 DETERMINATION

Place soln or aliquot contg 2–5 mg residue in 50 ml beaker; add 1 ml HCl (1+1) and enough H_2O to make 11 ml. Cover with watch glass and warm on steam bath. Add 2 ml phosphomolybdic acid soln dropwise with stirring, recover, and continue heating 20 min. Filter hot soln thru büchner into suction flask, and wash ppt and funnel with three 5 ml portions HCl (1+9), using policeman to scrub down walls of beaker. Aspirate dry. Wipe away any aq. soln at tip of büchner, change stopper to fit side arm test tube, and assemble. Dissolve ppt in three 5 ml portions acetone. Wash tip of büchner with few drops acetone, transfer to 25 ml vol. flask, and dil. to mark with acetone. Det. absorbance at 440 mμ against acetone, and det. mg caffeine from std curve. Calc. to g/100 ml or grains/bottle (1 grain = 64.8 mg).

8.036 PREPARATION OF STANDARD CURVE

Pipet 0, 1, 2, 3, 4, and 5 ml portions of the std caffeine soln into 50 ml beakers and proceed as in **8.035**, beginning "add 1 ml HCl (1+1) . . ." Plot absorbance against mg caffeine.

8.037 Alginates in Chocolate Beverage Products—Official—*See* **12.045**

SELECTED REFERENCES

(*1*) J. Agr. Research **33**, 301(1926); J. Assoc. Offic. Agr. Chemists **11**, 46, 505(1928).
(*2*) Ind. Eng. Chem. **15**, 732(1923); J. Assoc. Offic. Agr. Chemists **11**, 47, 505(1928).
(*3*) J. Assoc. Offic. Agr. Chemists **19**, 408(1936).
(*4*) Ibid. **16**, 420(1933); **19**, 75(1936).
(*5*) Ibid. **39**, 714(1956); **40**, 433(1957)

9. Beverages: Distilled Liquors

SPIRITS

9.001 Physical Examination—Procedure

Note and record following: (**a**) Color and depth of color; (**b**) odor—whisky, brandy, rum, etc., or foreign; (**c**) taste—whisky, brandy, rum, etc., or foreign.

Color—Official

9.002 DEFINITION

Whisky color units are defined as 10×absorbance at 430 mμ, measured in monochromatic light, of sample ½″ thick which has the spectral color characteristics of an av. whisky free of turbidity.

This definition applies only to absorbance values obtained with precise spectrophotometer with band width of 1 mμ or less at 430 mμ, and whose wavelength and photometer scales have been checked and corrected for inaccuracies by the methods recommended by NBS, in LC-929, Nov. 26, 1948.

Potassium Dichromate Calibration Method

9.003 PREPARATION OF STANDARD CURVE

Prep. solns of $K_2Cr_2O_7$ in $0.01N$ H_2SO_4 as follows:

COLOR UNIT	G/L	COLOR UNIT	G/L
1	0.0500	6	0.3000
2	0.1000	7	0.3500
3	0.1500	8	0.4000
4	0.2000	9	0.4500
5	0.2500	10	0.5000

Read absorbance of these solns in spectrophotometer at 430 mμ against H_2O, using same size cell as used in detns. If other than ½″ cell is used, convert reading to this size. Plot color units against absorbance or calc. av. factor for converting instrument reading to color units if straight line is obtained.

9.004 DETERMINATION

Place sample or sample dild with 50% alcohol in cell and det. absorbance against H_2O. Calc. color units, using factor or std curve.

Natural Coloring Matter (Organic-Soluble Color) (*1*)—Official

Spectrophotometric Method

9.005 APPARATUS

(**a**) *Spectrophotometer.—See* **9.002**.

(**b**) *Graduated cylinder.*—Cylindrical type of uniform diam., with pressed or molded base and ₮ stopper. Distance from base to top is 285–295 mm. To contain 50 ml at 20°, graduated in 0.2 ml with each fifth mark distinguished by longer line; numbered from bottom up at 2 ml intervals; error of graduations not >0.2 ml at any point. (Available from Scientific Glass Apparatus Co., Bloomfield, N. J.)

9.006 REAGENTS

(**a**) *n-Methyl propyl ketone.*—2-Pentanone; practical.

(**b**) *Saturated sodium chloride soln.*—USP or ACS NaCl.

(**c**) *Alcohol.*—MeOH, reagent grade, or alcohol, USP.

9.007 DETERMINATION

Pipet 20 ml whisky into cylinder, **9.005**(**b**). Add by pipet, in order, 10 ml satd NaCl soln, 0.5 ml HCl, and 10 ml Me propyl ketone. Immediately invert 10–15 times and let layers sep. Read vol. of org. layer within 1 hr and det. its absorbance at 430 mμ. If absorbance is too great or if solvent layer is cloudy, dil. aliquot to known vol. with either 50% MeOH or 50% EtOH and read absorbance.

9.008 CALCULATION

Example: If from 20 ml sample, org. layer of 16.1 ml was obtained which had absorbance of 0.420 in 1 cm cell after diln 1+1 with 50% alcohol: $(16.1 \times 0.420 \times 12.7 \times 2)/20 = 8.59$ color units (Lovibond number), where 12.7 is conversion factor to color units.

Specific Gravity (Apparent)—Official

9.009 APPARATUS

(**a**) *Constant temperature water bath.*

(**b**) *Pycnometers.*—100 ml and 50 ml (Fig. 18).

9.010 CALIBRATION

Fill thoroly cleaned pycnometer with recently distd H_2O, stopper, and immerse in constant temp. H_2O bath with bath level above graduation mark on pycnometer. After 30 min., remove stopper and with capillary tube adjust until bottom of meniscus is tangent to graduation mark. With small roll of filter paper, dry inside neck of pycnometer, stopper, and immerse in H_2O at room temp. 15 min. Remove pycnometer, dry, let stand 15 min., and weigh. Empty pyc-

nometer, rinse with acetone, and dry thoroly in air with suction. Let empty flask come to room temp., stopper, and weigh. Wt in air of contained H_2O = wt filled pycnometer − wt empty pycnometer.

9.011 DETERMINATION

Obtain wt sample as in **9.010.**

Sp. gr. in air = S/W, where S = wt sample, and W = wt H_2O.

Alcohol by Volume

From Specific Gravity by Pycnometer—Official

9.012 APPARATUS

(**a**) *Distillation apparatus.*—500 ml flask.

(**b**) *Liebig condenser.*—With jacket at least 400 mm long, inner tube i.d. 9 ± 1 mm, assembled vertically, with adapter attached with rubber tubing.

(**c**) *Connecting bulb.*—Iowa State type is convenient.

(**d**) *Connections.*—Use live rubber or ground-glass joints.

(**e**) *Electric or gas-operated heating unit.*

9.013 DETERMINATION

(**a**) *For samples containing 60% or less alcohol by volume.*—Calibrate 100 ml pycnometer, Fig. 18, as in **9.010,** at one of temps specified in **43.021.** Fill clean, dry pycnometer with sample and adjust to vol. at calibration temp. as in **9.010.**

Transfer contents of pycnometer to distg flask, just previously rinsed with cold H_2O and contg few glass beads or equiv. Rinse pycnometer 3 times, using total of 25 ml cold H_2O (40 ml for cordials or wines), and add rinse H_2O to flask. Place wet pycnometer so that adapter extends just into bulb. Surround pycnometer with ice or ice-H_2O. Complete connections and pass thru H_2O-jacket rapid stream of H_2O kept at not >25° at outlet. Distill ca 96 ml at uniform rate in not <30 nor >60 min., using longer periods of time for higher percentages of alcohol. Remove and stopper pycnometer, mix distillate by swirling, and wash down with H_2O any drops that may be above graduation mark. Immerse in constant temp. bath at calibration temp. and after 30 min. carefully dil. to vol., with aid of capillary tube, by adding H_2O previously boiled and cooled to same temp. Det. sp. gr. of distillate as in **9.011.** Obtain corresponding % alcohol by vol. from **43.021.** (This result is % alcohol by vol. at 15.56° (60°F).)

(**b**) *For samples containing more than 60% alcohol by volume.*—Proceed as in (**a**) with following changes: Calibrate 100 ml and 50 ml pycnometers, Fig. 18, at 15.56°, fill 50 ml pycnometer with sample, and adjust to vol. at 15.56°. Add 50 ml cold H_2O to distg flask before transfer of sample

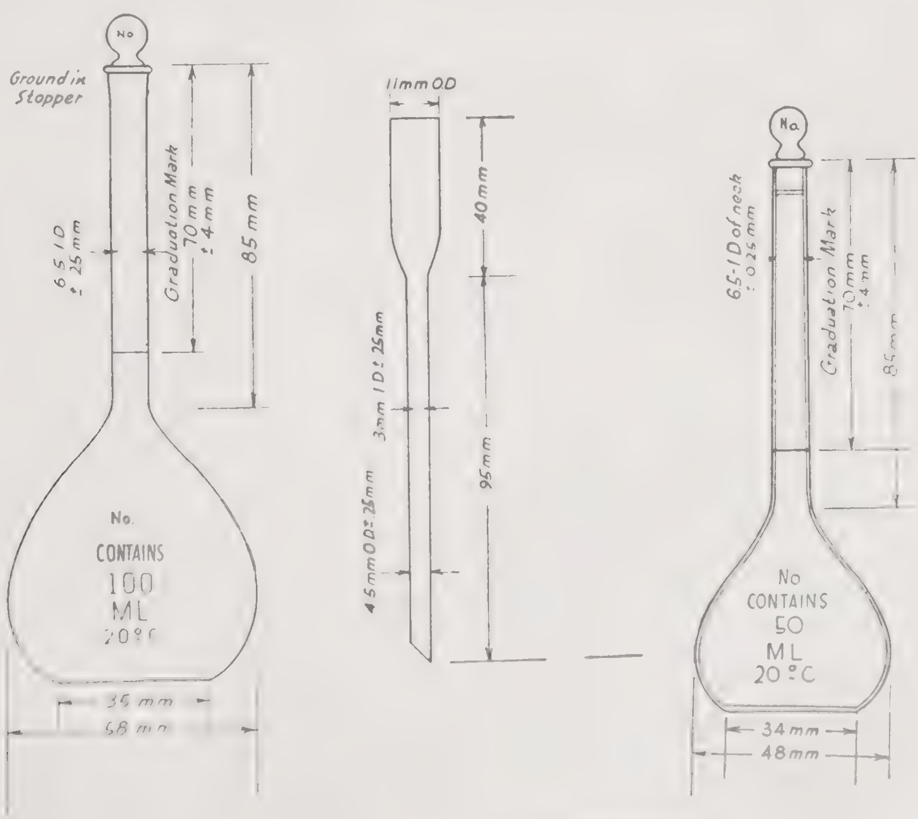

FIG. 18. 100 ML AND 50 ML PYCNOMETERS

and collect distillate in 100 ml pycnometer. Obtain sp. gr. of distillate, and from table, **43.021**, obtain % alcohol by vol. in distillate. Calc. as follows: % alcohol by vol. in sample at 15.56° $= D \times W/W'$; where $D =$ % alcohol by vol. in distillate at 15.56°; $W =$ wt H_2O at 15.56° in 100 ml pycnometer; and $W' =$ wt H_2O at 15.56° in 50 ml pycnometer.

From Specific Gravity by Hydrometer (2)—Official

(Applicable to spirits contg not >600 mg ext./100 ml.)

9.014 APPARATUS

(a) *Hydrometer.*—Graduated to 0.1 or 0.2° proof, with calibration corrections.

(b) *Thermometer.*—Graduated to 0.25 or 0.5°F, with calibration corrections.

(c) *Cylinder.*—Clear glass, 2.5″ diam., 14″ high.

(d) *Metal clips.*—To hold thermometer in cylinder.

9.015 DETERMINATION

Clean and dry hydrometer before use. Let hydrometer, thermometer, cylinder, and sample come to room temp. Rinse cylinder, contg thermometer held in place by spring frame clip, 2 or 3 times with portion of sample. Fill cylinder to desired level with sample, holding cylinder at angle of ca 45° to reduce agitation and air bubbles. (After hydrometer is inserted, liquid level should be slightly below rim of cylinder.) Place palm of hand over top of cylinder and slowly invert 3 or 4 times to equalize temps of liquid and cylinder. Wipe off any liquid on outside of cylinder. (Do not place hands on cylinder in such way as to warm liquid inside.) Insert hydrometer in liquid; then raise and lower hydrometer bulb from top to bottom 5 or 6 times to temper and distribute slight temp. changes thruout liquid. Keep hydrometer bulb in liquid, dry stem, and let hydrometer come to rest without wetting more than few tenths degrees of exposed stem.

Read hydrometer, then thermometer. To read hydrometer scale, place eye slightly below plane of surface of liquid, and then slowly raise head, keeping eye perpendicular to hydrometer, until surface flattens from ellipse into straight line. Take point where this line intersects hydrometer scale as reading of hydrometer.

Raise hydrometer slightly above its point of rest and again let it come to rest in liquid. Read hydrometer and thermometer again to verify original readings. Read hydrometer to nearest 0.02° and thermometer to nearest 0.1°. Remove and dry hydrometer. Re-invert cylinder and contents several times (with thermometer left in place) to thermally equilibrate system. Retemper hydrometer, dry stem, and again read hydrometer and thermometer. Apply calibration corrections for both hydrometer and thermometer. Calc. true % of proof from Table No. 1 of the U. S. Treasury Department Gauging Manual, 1950 (prepd by NBS and based on information published in Bull. NBS, Vol. 9, No. 3, pp. 327–474, Oct. 15, 1913). Average calcd values if they agree within 0.1° proof; otherwise take addnl readings and average.

Det. ext. as in **9.022** and for every 100 mg ext. /100 ml add 0.4° proof to apparent proof.

9.016 *From Refraction—Official*

Measure 25 ml sample into distn flask, noting temp; dil. with 100 ml H_2O, distill nearly 100 ml, dil. to vol. at same temp., and det. immersion refractometer reading. Obtain corresponding % alcohol from **43.022**.

Williams Field Test (3)—Procedure

9.017 APPARATUS

Williams tube.—*See* Fig. 19. Clean frequently and dry.

9.018 REAGENT

(a) *Dilute hydrochloric acid.*—Dil. 10 ml HCl to 100 ml with H_2O.

(b) *Solvent.*—Mix 70 ml Pentasol (synthetic

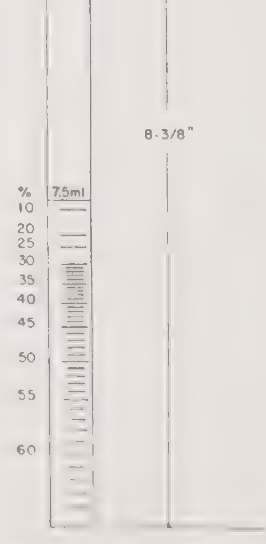

FIG. 19.—WILLIAMS TUBE

amyl alcohol), 28 ml toluene, and 2 ml of the dil. HCl. Shake well until acid completely dissolves.

9.019 DETERMINATION

Place sample in tube, accurately adjusting bottom of meniscus to coincide with 7.5 ml mark. Remove any excess sample on sides of tube above 7.5 ml mark with swab or roll of filter paper. Add 7.5 ml reagent to 15 ml mark. Stopper tube and invert number of times to mix intimately. Stand tube upright and let sep. When sepn is complete, shake down globules of lower soln that adhere to sides by rotating tube, and stopper. When settling and drainage are complete, read % alcohol (by vol.) where meniscus between the 2 layers falls on calibration mark. Repeat operation of mixing and settling, and read again.

9.020 TEMPERATURE CORRECTION

Correct for effects of temp. and alcohol concn according to following table.

Temperature (°F) correction factors

%	60°	62°	64°	66°	68°	70°	72°	74°	76°	78°
43	+0.5	+0.3	+0.2	0.0	−0.1	−0.2	−0.4	−0.5	−0.7	−0.8
43.4	+0.5	+0.4	+0.2	+0.1	0.0	−0.2	−0.3	−0.5	−0.6	−0.7
44	+0.6	+0.4	+0.3	+0.2	0.0	−0.1	−0.3	−0.4	−0.5	−0.7
45	+0.7	+0.5	+0.4	+0.3	+0.1	0.0	−0.2	−0.3	−0.4	−0.6
46	+0.8	+0.7	+0.5	+0.4	+0.2	+0.1	0.0	−0.2	−0.3	−0.5
47	+0.9	+0.8	+0.6	+0.5	+0.3	+0.2	+0.1	−0.1	−0.2	−0.4
47.5	+1.0	+0.8	+0.7	+0.5	+0.4	+0.3	+0.1	0.0	−0.2	−0.3
48	+1.1	+0.9	+0.7	+0.6	+0.4	+0.3	+0.2	0.0	−0.1	−0.2
49	+1.1	+1.0	+0.8	+0.7	+0.6	+0.4	+0.3	+0.1	0.0	−0.1
50	+1.2	+1.1	+0.9	+0.8	+0.7	+0.5	+0.4	+0.3	+0.1	0.0

%	80°	82°	84°	86°	88°	90°	92°	94°	96°	98°
43	−0.9	−1.1	−1.2	−1.4	−1.5	−1.6	−1.8	−1.9	−2.0	−2.2
43.4	−0.9	−1.0	−1.2	−1.3	−1.4	−1.6	−1.7	−1.9	−2.0	−2.2
44	−0.8	−0.9	−1.1	−1.2	−1.4	−1.5	−1.6	−1.8	−1.9	−2.1
45	−0.7	−0.8	−1.0	−1.1	−1.3	−1.4	−1.5	−1.7	−1.8	−2.0
46	−0.6	−0.7	−0.9	−1.0	−1.2	−1.3	−1.4	−1.6	−1.7	−1.8
47	−0.5	−0.6	−0.8	−0.9	−1.0	−1.2	−1.3	−1.5	−1.6	−1.7
47.5	−0.4	−0.6	−0.7	−0.8	−1.0	−1.1	−1.3	−1.4	−1.5	−1.7
48	−0.4	−0.5	−0.6	−0.8	−0.9	−1.1	−1.2	−1.3	−1.5	−1.6
49	−0.3	−0.4	−0.5	−0.7	−0.8	−1.0	−1.1	−1.2	−1.4	−1.5
50	−0.2	−0.3	−0.4	−0.6	−0.7	−0.9	−1.0	−1.1	−1.3	−1.4

Example: Indicated % alcohol from tube: 48.0% at 90°F. Correction factor from table is −1.1; 48.0 − 1.1 = 46.9%.

9.021 **Alcohol by Weight—Official**

Weigh accurately 40–50 g sample, using clean, dry 50 ml pycnometer, Fig. 18, or other closed vessel. (If alcohol is 60% or less by vol., the 100 ml sample of **9.013(a)** may be weighed and used.) Transfer to 500 ml distn flask contg 50 ml H_2O and few clean glass beads or equiv. Rinse pycnometer 3 times, bringing contents of distn flask to ca 125 ml. Distill, and det. % alcohol by vol. in distillate as in **9.013(a)**. Ascertain corresponding % alcohol by wt in distillate from table, **43.023**. Multiply result by wt distillate and divide by wt sample.

9.022 **Extract—Official**

Weigh, or measure at 20°, 25–100 ml sample, evap. to dryness on steam bath, dry 30 min. at 100°, cool in desiccator 30 min., and weigh.

9.023 **Ash—Official**

Proceed as in **29.012** or **29.013**, using residue from **9.022**.

9.024 **Phosphorus—Official**—*See* **11.025**

9.025 **Total Acids—Official**

Neutralize ca 250 ml boiled H_2O in porcelain evapg dish (7½″ dish is convenient). Add 25 ml sample and titr. with 0.1*N* NaOH, using ca 2 ml phthln.

9.026 **Fixed Acids—Official**

Evap. 25–50 ml sample to dryness in Pt dish on steam bath and dry 30 min. in oven at 100°. Dissolve and transfer residue with several portions of neutral alcohol of ca same proof as sample,

using 25–50 ml in all, to porcelain dish contg ca 250 ml neutralized boiled H_2O. Titr. with 0.1N NaOH, using 10 ml buret graduated in 0.05 ml and same quantity of indicator as in **9.025**.

9.027 Volatile Acids—First Action

Volatile acids = total acids from **9.025** – fixed acids from **9.026**.

Esters and Aldehydes—Official

9.028 REAGENTS

(**a**) *Sodium thiosulfate std soln.*—0.05N. Prep. by dilg 0.1N soln, **42.035**.

(**b**) *Iodine soln.*—Approx. 0.05N.

(**c**) *Sodium bisulfite soln.*—Approx. 0.05N. (Deterioration is retarded if soln contains ca 10% alcohol; do not use after ca 1 week.)

(**d**) *Oxidizing soln.*—Dissolve 100 g $K_2Cr_2O_7$ in 900 ml H_2O and add 100 ml H_2SO_4.

9.029 PREPARATION OF SAMPLE

To 200 ml sample in 500 ml erlenmeyer, add ca 35 ml H_2O and few grains SiC (Carborundum). Distill slowly into 200 ml vol. flask until distillate is nearly at mark. Dil. to mark and mix.

9.030 DETERMINATION OF ESTERS (*4*)

Transfer 100 ml distillate to 500 ml flask, neutralize free acid, add measured excess 0.1N NaOH, connect flask with air-cooled condenser ca 2 ft long, heat 2 hr on steam bath, let cool, and titr. excess alkali. Reject detns in which excess 0.1N alkali is <2 ml. or is >10 ml. Calc. esters as EtOAc.

9.031 DETERMINATION OF ALDEHYDES

Place remainder of distillate from **9.029** in 500 ml flask, add ca 100 ml H_2O and excess $NaHSO_3$ soln, and let stand ca 30 min., shaking occasionally. (Excess $NaHSO_3$ should be equiv. of ca 25 ml I soln.) Add excess I soln, and titr. this excess with the $Na_2S_2O_3$ soln. Run blank contg same quantities of I soln and bisulfite soln as used in sample. Difference between titrns in ml $Na_2S_2O_3$ soln $\times 1.1$ = mg acetaldehyde in sample.

Fusel Oil (*5*)—First Action

9.032 REAGENTS

(**a**) *p-Dimethylaminobenzaldehyde soln.*—In 100 ml vol. flask dissolve 1 g *p*-dimethylaminobenzaldehyde in mixt. of 5 ml H_2SO_4 and 90 ml H_2O, and dil. to mark with H_2O.

(**b**) *Isobutyl alcohol.*—Eastman X-303 (Fusel oil std).

(**c**) *Isoamyl alcohol.*—Eastman X-18 (Fusel oil std).

(**d**) *Ethyl alcohol.* Redistd middle 50% fraction.

(**e**) *Synthetic std fusel oil.*—Weigh 2 g isobutyl alcohol and 8 g isoamyl alcohol into 1 L vol. flask and dil. to mark with H_2O. Pipet two 10 ml portions into 100 ml vol. flasks and dil. to mark, one with H_2O and other with alcohol. Prep. working stds for products in range of 0–170 proof contg 1.0–6.0 g synthetic fusel oil/100 L by dilg 1.0–6.0 ml aliquots of aq. std soln to 100 ml with alc. soln of proof expected for dild sample when pipeted into analysis tube. Prep. similar working stds for products in range of 170–190 proof by dilg 1.0–6.0 ml aliquots of alc. std soln to 100 ml with alc. soln of proof of sample or its diln.

9.033 PREPARATION OF SAMPLES

(Aged, blended and rectified products, whiskies, brandies, rums, vodka, and liqueurs require distn.)

Determination of true proof of sample.—Det. alcohol as in **9.013**.

For samples contg >6 g fusel oil/100 L, dil. distd sample with H_2O to concn of 2.0–5.0 g fusel oil/100 L. Dil. 5 ml brandies, rum, and blended whiskies to 100 ml; dil. 5 ml heavy brandies, rums, and straight whiskies to 250 ml.

9.034 DETERMINATION

Pipet 2 ml aliquots of sample or dild sample, 2 ml H_2O (for reagent blank), and 2 ml aliquots of stds into 15×150 mm stoppered or covered test tubes. Stopper or cover tubes, and place in rack, then in ice bath. Pipet 1 ml *p*-dimethylaminobenzaldehyde soln into each tube, shake, and replace in ice bath for 3 min. With tubes still in ice bath, add 10 ml H_2SO_4 from buret down side of each tube. Shake tubes individually and replace in ice bath for 3 min. Transfer rack of tubes from ice bath to boiling H_2O bath and boil 20 min. Transfer tubes to ice bath for 3–5 min., then to room temp. bath. Read % transmittance of developed color of samples and stds on spectrophotometer at 530–535 mμ against reagent blank as reference. (Use same wavelength for both stds and unknowns.) Plot g fusel oil/100 L on linear scale as abscissa against % T as ordinate on log scale of semilog paper. Convert % transmittance of samples to g fusel oil/100 L from std curve. If diln was used, multiply g fusel oil/100 L found by diln factor to obtain g fusel oil/100 L in original sample. Analyze 2 levels of stds with each series of unknowns.

Precision expected: Whiskies and brandies, ±5%; rum, ±8%; gin, vodka, spirits, ±0.4 g/100 L.

Furfural (*6*)—First Action

9.035 REAGENT

Furfural std soln.—Redistill furfural thru short packed fractionating column at atmospheric pres-

sure, and collect fraction boiling at 161.2° (uncorrected). Weigh 1 ml redistd furfural into 100 ml vol. flask and dil. to mark with alcohol. Pipet 5 ml of this soln into 500 ml vol. flask and dil. to mark with 50% alcohol. (Concn, ca 116 mg/L.) Concd soln retains strength, but dil. soln does not.

9.036 DETERMINATION

Pipet 25 ml distd spirits into volatile acid distn flask, Fig. 22, with ₸ joints and steam distill until 200 ml collects. If haze is present in distillate, dil. with known vol. alcohol. Det. absorbance, A, at 277 mμ.

Det. absorbances of std solns of furfural contg 0, 1, 2, 3, 4, and 5 mg furfural/L. Plot std curve or calc. av. absorbance of 1 mg furfural/L, A' (ca 0.15). Furfural, mg/L $= (A/A') \times F$, where F is diln factor (vol. final soln on which absorbance is detd/vol. sample).

Detection of Acetone, Other Ketones, Isopropanol, and Tertiary Butyl Alcohol—First Action

9.037 REAGENT

Mercuric sulfate soln.—Mix 5 g yellow HgO with 40 ml H_2O and add, with stirring, 20 ml H_2SO_4 and 40 ml H_2O. Stir until completely dissolved.

9.038 DETERMINATION

To 2 ml distillate, **9.029,** add 3 ml H_2O and 10 ml of the $HgSO_4$ soln. Heat on boiling H_2O bath 3 min. White or yellow ppt forming within 3 min. indicates presence of acetone, another ketone, or *tert*-butyl alcohol. Disregard any ppt forming after 3 min. on boiling H_2O bath.

If no ppt forms, test for isopropanol as follows: Place 8 g CrO_3 in 100 ml Kohlrausch flask, and add 15 ml H_2O and 2 ml H_2SO_4. Connect flask with reflux condenser and add 5 ml sample very slowly thru condenser. Boil under reflux condenser 30 min.; then cool and distill off 2 ml, collecting distillate in 10 ml graduated cylinder. Add 3 ml H_2O and 10 ml of the $HgSO_4$ soln, and proceed as above.

9.039 Sugars—Official—*See* Chap. 29

Methanol

Chromotropic Acid Colorimetric Method (7)—Official

9.040 REAGENTS

(a) *Potassium permanganate soln.*—Dissolve 3.0 g $KMnO_4$ and 15.0 ml H_3PO_4 in 100 ml H_2O. Prep. monthly.

(b) *Sodium salt of chromotropic acid (sodium 1,8-dihydroxynaphthalene-3,6-disulfonate).*— 5% soln. Filter if not clear. Prep. weekly.

9.041 PURIFICATION OF CHROMOTROPIC ACID

If absorbance of blank is $>$ca 0.05, purify reagent as follows:

Dissolve 10 g chromotropic acid or its salt in 25 ml H_2O. (Add 2 ml H_2SO_4 to aq. soln of the salt to convert it to free acid.) Add 50 ml MeOH, heat just to boiling, and filter. Add 100 ml isopropyl alcohol to ppt free chromotropic acid. (Add more isopropyl alcohol to increase yield of purified acid.)

9.042 PREPARATION OF SAMPLE

Dil. or adjust sample to total alc. concn of 5–6%. Using 50 ml sample, distill thru simple still, collecting 40 ml distillate. Dil. to 50 ml with H_2O. (If alcohol has been detd previously, distillate may be adjusted to 5–6% alc. concn and used for this test.) If $>$0.05% MeOH by vol. is present, dil. to ca that concn with 5.5% alcohol. For samples contg $<$0.05% MeOH, measure 200 ml into efficient fractionating still, place system under total reflux 15 min., and then slowly distill at high rate of reflux (at least 20:1). Collect 10 ml distillate and dil. to 160 ml with H_2O.

9.043 DETERMINATION

Pipet 2 ml $KMnO_4$ soln into 50 ml vol. flask. Chill in ice bath, add 1 ml chilled dild sample, and let stand 30 min. in ice bath. Decolorize with little dry $NaHSO_3$ and add 1 ml chromotropic acid soln. Add 15 ml H_2SO_4 slowly with swirling and place in hot (60–75°) H_2O bath 15 min. Cool, add enough H_2O to bring approx. to 50 ml mark, mix, and dil. to vol. with H_2O at room temp. Read absorbance at 575 mμ, using reagent blank of 5.5% alcohol treated similarly as reference. Treat *std MeOH soln* contg 0.025% by vol. MeOH in 5.5% alcohol simultaneously in same manner, and read absorbance. (Temp. of std and sample should be within 1° since temp. affects depth of color.)

Calc. quantity of MeOH in sample as follows: % MeOH $= (A/A') \times 0.025 \times F$, where A = absorbance of sample, A' = absorbance of std MeOH, and F = diln factor of sample.

Example: Sample was dild 25 times; absorbance of sample = 0.421; absorbance of std MeOH = 0.368. Then $(0.421/0.368) \times 0.025 \times 25 = 0.715\%$.

(If color of sample is too intense, dil. with H_2SO_4-alcohol blank prepd as above. Not $>$3-fold diln is permitted, as ratio of chromotropic acid to HCHO is too low if diln is greater.)

9.044 *Immersion Refractometer Method (8)—Official*

Det. Zeiss immersion refractometer reading at 17.5° of distillate obtained in detn of alcohol. If,

on reference to table, 9.045, refractometer reading shows sp. gr. agreeing with that obtained in alcohol detn, 9.013, it may be assumed that no MeOH is present. Low refractometer reading indicates presence of appreciable quantity of MeOH. If absence from the soln of refractive substances other than H_2O and the alcohols is assured, this difference in refraction is conclusive evidence of presence of MeOH.

Addn of MeOH to alcohol decreases refractive index in direct proportion to quantity added; hence quant. calcn is made by interpolation in table, **9.045**, of figures for pure alcohol and MeOH of same sp. gr. as sample.

Example.—Distillate has sp. gr. at 15.56° of 0.9625 and refractometer reading at 17.5° of 43.1. By interpolation in table, readings for alcohol and MeOH at this gravity are 65.2 and 31.7, resp., and difference is 33.5; $65.2-43.1=22.1$; $(22.1\div33.5)\times100=66.0$, showing 66.0% of total alcohol present is MeOH.

g-s. cylinder, and wash until filtrate vol. is ca 15 ml. Dil. filtrate with absolute alcohol to 25 ml. Mix thoroly, and either compare with original material in photometer, using ½" cell and filter transmitting light at 460 mμ, or examine in Lovibond Tintometer. If spectrophotometer is used, make readings at 430 mμ and calc. as in **9.002**. From these results calc. % color insol. in H_2O (100%—color observed).

Artificial Colors

9.047 *Marsh Test—First Action*

To 10 ml sample in 20 ml test tube add enough freshly shaken *Marsh reagent* (100 ml amyl alcohol, 3 ml H_3PO_4, and 3 ml H_2O) to nearly fill tube, and shake several times. Let layers sep. Color in lower layer indicates that sample has been colored with caramel, coal-tar dye, or extractive material from uncharred white oak chips.

9.045 *Scale readings on Zeiss immersion refractometer at 17.5°, corresponding to specific gravities of ethyl and methyl alcohol solutions*

SP. GR. 15.56°/15.56°	SCALE READINGS		DIFFERENCES	SP. GR. 15.56°/15.56°	SCALE READINGS		DIFFERENCES
	ETHYL ALCOHOL	METHYL ALCOHOL			ETHYL ALCOHOL	METHYL ALCOHOL	
1.0000	15.0	15.0	0.0	.9720	51.5	27.0	24.5
.9990	15.8	15.3	0.5	.9710	53.0	27.5	25.5
.9980	16.6	15.6	1.0	.9700	54.6	28.1	26.5
.9970	17.5	15.9	1.6	.9690	56.1	28.7	27.4
.9960	18.5	16.2	2.3	.9680	57.6	29.2	28.4
.9950	19.4	16.5	2.9	.9670	59.1	29.6	29.5
.9940	20.4	16.9	3.5	.9660	60.6	30.1	30.5
.9930	21.4	17.2	4.2	.9650	62.0	30.6	31.4
.9920	22.5	17.5	5.0	.9640	63.3	31.0	32.3
.9910	23.6	17.9	5.7	.9630	64.6	31.5	33.1
.9900	24.7	18.2	6.5	.9620	65.8	31.9	33.9
.9890	25.9	18.6	7.3	.9610	67.0	32.4	34.6
.9880	27.1	19.0	8.1	.9600	68.1	32.8	35.3
.9870	28.4	19.5	8.9	.9590	69.2	33.3	35.9
.9860	29.6	19.9	9.7	.9580	70.2	33.7	36.5
.9850	31.0	20.4	10.6	.9570	71.2	34.1	37.1
.9840	32.4	20.8	11.6	.9560	72.1	34.5	37.6
.9830	33.8	21.3	12.5	.9550	73.0	34.9	38.1
.9820	35.2	21.8	13.4	.9540	73.8	35.3	38.5
.9810	36.7	22.3	14.4	.9530	74.6	35.6	39.0
.9800	38.3	22.8	15.5	.9520	75.4	35.9	39.5
.9790	39.9	23.4	16.5	.9510	76.2	36.2	40.0
.9780	41.5	24.0	17.5	.9500	76.9	36.5	40.4
.9770	43.1	24.5	18.6	.9490	77.6	36.8	40.8
.9760	44.8	25.0	19.8	.9480	78.3	37.0	41.3
.9750	46.5	25.5	21.0	.9470	79.0	37.3	41.7
.9740	48.2	26.0	22.2	.9460	79.7	37.6	42.1
.9730	49.8	26.5	23.3				

Scale readings are applicable only to instruments calibrated in arbitrary scale units proposed by Pulfrich, *Z. angew. Chem.*, 1899, p. 1168. According to this scale, $14.5=1.33300$, $50.0=1.34650$, and $100.0=1.36464$. If instrument used is calibrated in other arbitrary units, refractive index corresponding to observed reading can be converted into equivalent Zeiss reading by referring to **43.022**.

9.046 **Water-Insoluble Color—First Action**

Evap. 25 ml sample spontaneously to dryness. Take up in just enough cold H_2O, filter into 25 ml

In absence of any color, test 10 ml in same manner, using enough fusel oil, amyl alcohol, or Pentasol to nearly fill tube and shaking several times. Deeply colored lower layer indicates coal-tar dye. Ascertain its identity as in Chap. **35**. To

confirm caramel apply one or more of following tests:

9.048 *Mathers Test—Official—See* **11.040**

9.049 *Cyclohexanol Test—First Action*

To 10 ml sample dild to <80° proof in 20 ml test tube add enough *cyclohexanol reagent* (mixt. of 50 vols each of cyclohexanol and Me propyl ketone and 3 vols each of H_3PO_4 and H_2O) to nearly fill tube, and invert several times. Let layers sep. Color in lower layer indicates sample contains caramel or coal-tar dye.

9.050 Coal-Tar Colors—First Action— *See* Chap. **35**

Tannin *(9)***—First Action**

9.051 REAGENTS

(**a**) *Folin-Denis reagent.*—To 750 ml H_2O add 100 g $Na_2WO_4.2H_2O$, 20 g phosphomolybdic acid, and 50 ml H_3PO_4. Reflux 2 hr, cool, and dil. to 1 L.

(**b**) *Sodium carbonate satd soln.*—To each 100 ml H_2O add 35 g anhyd. Na_2CO_3, dissolve at 70–80°, and let cool overnight. Seed supersatd soln with crystal of $Na_2CO_3.10H_2O$, and after crystn filter thru glass wool.

(**c**) *Tannic acid std soln.*—Dissolve 100 mg tannic acid in 1 L H_2O. Prep. fresh soln for each detn.

9.052 PREPARATION OF STANDARD CURVE

Pipet 0 to 10 ml aliquots of the std tannic acid soln into 100 ml vol. flasks contg 75 ml H_2O. Add 5 ml Folin-Denis reagent and 10 ml Na_2CO_3 soln, and dil. to mark with H_2O. Mix well and det. absorbance after 30 min. at 760 mμ. Plot absorbance against mg tannic acid/100 ml.

9.053 DETERMINATION

Using 1 ml sample, det. absorbance as in **9.052** and obtain mg tannic acid/100 ml from std curve. If absorbance is too great, repeat detn on 1+4 diln of sample. Samples treated as above may be compared in Nessler tubes against freshly prepd tannic acid stds treated in same manner.

CORDIALS AND LIQUEURS

9.054 Physical Examination—Procedure

Note and record following: (*a*) Appearance, whether bright or turbid and presence of sediment; (*b*) color and depth of color; (*c*) odor; (*d*) taste.

9.055 Specific Gravity—Official—*See* **9.011**

9.056 Alcohol—Official

(**a**) *By weight.—See* **9.021**.

(**b**) *By volume.—See* **9.013**. Use pycnometer calibrated at 15.56°.

Methanol—Official

9.057 PREPARATION OF SAMPLE

Measure sample contg 20–25 ml absolute alcohol into distg flask, add enough H_2O to make total ca 100 ml, and distill, collecting ca 50 ml distillate. To distillate add 4 g NaCl for each 10 ml H_2O and let stand several hr for complete satn.

Transfer to separator, using ca 10 ml satd NaCl soln to wash out container, and shake with 25 ml petr. ether. When sepn is complete, transfer aq. soln to second separator contg 25 ml petr. ether; shake, and transfer aq. soln to third separator, also contg 25 ml petr. ether; shake, and when sepn is complete, drain aq. soln into 200 ml distg flask. Meanwhile add 25 ml satd NaCl soln to first separator and follow sample thru with this soln, finally adding washings to sample soln in distg flask. Repeat this operation with second 25 ml portion satd NaCl soln, finally adding this also to distg flask. Distill mixt. into 50 ml vol. flask, using suitable adapter. After 48–49 ml distills, disconnect app., fill flask to mark with H_2O, mix, and det. MeOH as in **9.043** or **9.044**.

9.058 Aldehydes—Official

Measure 100–200 ml sample into distn flask. If solid content is 25 g/100 ml or less, add 12.5–25 ml H_2O; if >25 g/100 ml, add 5 ml H_2O for each 10 g solid matter present; distill slowly, collecting vol. distillate equal to that of sample, and proceed as in **9.031**.

9.059 Furfural—Official

Dil. portion prepd distillate, **9.058**, to 50 ml with furfural-free alcohol (USP is usually satisfactory), 50% by vol. Add 2 ml *colorless aniline* and 0.5 ml HCl, sp. gr. 1.125, and keep 15 min. in H_2O bath at ca 15°. Compare color developed with stds treated in same way, contg 0.0, 0.05, 0.1, 0.15, 0.2, 0.25, and 0.3 mg furfural, prepd as in **9.035**.

9.060 Fusel Oil—Official

Using 50 ml prepd distillate, **9.058**, proceed as in **9.034**.

9.061 Total Solids

(**a**) *From specific gravity of dealcoholized sample (First Action).*—Transfer residue from alcohol detn, **9.056(b)**, to original pycnometer with H_2O, dil. to mark with H_2O at 15.56°, and mix. Adjust temp. of pycnometer and contents to 20°; adjust meniscus to mark, using capillary tube or narrow strips of filter paper to remove any excess liquid while in the 20° bath. Weigh, and calc. sp. gr. of liquid. From **43.003** ascertain % dry substance and corresponding sp. gr. at 20°/4°. Sp. gr. at

20°/4° × % dry substance = total solids (g/100 ml).

(**b**) *By evaporation* (*Official*).—Fill 25 ml vol. flask with sample at 20°, and adjust meniscus by means of capillary tube or narrow strips of filter paper while flask is immersed in bath held at same temp. ca 30 min. Quantitatively transfer contents of flask to 100 ml vol. flask with H_2O and fill to mark with H_2O at convenient temp. At same temp. pipet 10 ml dild sample into dish contg sand and dry as in **29.008.** Wt residue × 40 = total solids (g/100 ml).

(**c**) *From refractive index of dealcoholized sample* (*Official*).—Restore residue from alcohol detn to original vol. by evapg or dilg as necessary. Det. refractometer reading of soln at 20° and obtain corresponding % dry substance. From **43.003** det. sp. gr. corresponding to % dry substance found and multiply by % dry substance to obtain g total solids/100 ml sample. To obtain % total solids, divide total solids/100 ml by sp. gr., **9.011.**

9.062 Glycerol—Official

(**a**) *Products containing 5 g/100 ml or less of total solids.*—*See* **11.012**(**a**) or (**b**).

(**b**) *Products containing more than 5 g/100 ml of total solids.*—Into porcelain dish measure sample (not >100 ml) contg 25 g or less solid matter and evap. on steam bath to remove alcohol. Transfer to 500 ml erlenmeyer, using such quantity of H_2O that final vol. will be ca 100 ml, and proceed as in **11.013.**

9.063 Sucrose—Official

(**a**) *By polarization.*—Pipet, into evapg dish, vol. sample equiv. to 52 g as calcd from sp. gr., **9.011,** and exactly neutralize with 1*N* NaOH, calcg quantity required from acidity, **9.070.** Evap. on steam bath to remove alcohol, transfer to 200 ml vol. flask, and proceed as in **29.025** or **29.026,** beginning "add necessary clarifying reagent . . . " in **29.025**(**a**).

(**b**) *By reducing sugars before and after inversion.*—Approximate sugar content of sample from total solids, **9.061,** and pipet sample contg 5–7 g sugars into porcelain dish; exactly neutralize with 1*N* NaOH soln, calcg quantity required from acidity, **9.070,** and evap. on steam bath to remove alcohol. Transfer to 200 ml vol. flask, clarify with neutral $Pb(OAc)_2$ soln, **29.021**(**d**), remove excess Pb with K oxalate, and proceed as in **29.032,** using **29.039** for detn of reducing sugars.

9.064 Ash—Official

Proceed as in **29.012** or **29.013,** using 25 ml sample.

9.065 Soluble and Insoluble Ash—Official

Using ash from **9.064,** proceed as in **29.015.**

9.066 Alkalinity of Soluble Ash—Official

Using sol. ash from **9.065,** proceed as in **29.016.**

9.067 Alkalinity of Insoluble Ash—Official

Using insol. ash from **9.065,** proceed as in **29.017.**

9.068 Phosphorus—Official

Using ash obtained in **9.064,** det. P_2O_5 as in **11.023** or **11.025.**

9.069 Caramel—Official—*See* 11.040–11.041

9.070 Total Acidity—First Action

Place ca 600 ml H_2O in 800 ml beaker, add ca 1 ml phthln, and titr. to pink color with 0.1*N* NaOH. Add 10–20 ml sample (unless this quantity gives soln such deep color that it will obscure end point, in which case 5 ml may be used) and titr. to pink comparable to that of soln before sample was added. Calc. acidity as g/100 ml sample in terms of predominating acid present in sample.

9.071 Characteristic Acids—Preparation of Sample—Procedure

Use sample contg not >30 g solids and not >200 mg acid to be detd, as calcd from acidity; evap. to ca 30 ml and treat as in **9.072–9.075.**

9.072 Tartaric Acid—First Action

Designate as *A* the ml 1*N* alkali required to neutralize sample, add *A* + 3 ml 1*N* NaOH, heat to ca 60°, and let stand overnight. Add *A* + 6 ml 1*N* H_2SO_4 and continue as in **20.044,** beginning "Transfer adjusted sample into 250 ml vol. flask . . . "

9.073 Citric Acid—First Action

Transfer adjusted sample to 250 ml vol. flask, using enough H_2O to make total vol. 70 ml, and continue as in **20.047,** beginning "Add 2 ml 1*N* HNO_3 . . . "

9.074 Total Malic Acid (Laevo and Inactive)—First Action

Proceed as in **9.072** to obtain filtrate and washings from KH tartrate; then evap. this soln to ca 15 ml and continue as in **20.053,** beginning "Transfer with small amount of H_2O . . . "

9.075 Laevo Malic Acid—First Action

Proceed as in **9.072** to obtain filtrate and washings from KH tartrate; then evap. to ca 5 ml on steam bath and proceed as in **20.061,** beginning "Cool, add NaOH (1 + 1) . . . "

9.076 Volatile Esters—Official

Measure 100–500 ml sample into distg flask and steam distill as in **8.026,** collecting vol. distillate

at least twice as great as vol. alcohol contained in sample. (If detn **9.077** is to be made, use 500 ml sample.) Disconnect app. and wash out condenser with little H_2O. Add ca 1 ml phthln, and titr. to pink color that persists >1 min., using $0.1N$ NaOH or KOH. Add to soln measured excess of 25–50 ml $0.1N$ alkali, reflux 1 hr, cool, and titr. excess of alkali with $0.1N$ H_2SO_4. Calc. number of ml $0.1N$ alkali used in saponification of esters as EtOAc. 1 ml $0.1N$ alkali = 8.8 mg EtOAc.

9.077 Gamma Undecalactone (Qualitative Test) (*10*)—Official

(Peach and apricot cordials)

Make soln obtained in **9.076** distinctly alk. and evap. to dryness on steam bath. Take up residue in ca 25 ml H_2O, transfer to separator, acidify with H_2SO_4 (1+1), let stand 10 min. to permit lactones to form, and ext. with three ca 20 ml portions ether. Combine ether exts and wash by shaking with three 10 ml portions $1N$ Na_2CO_3 soln. Let ether soln evap. spontaneously in small beaker. To residue add few drops *$N_2H_4.H_2O$ soln* (42% in H_2O) and mix thoroly; if white solid matter seps in few min., gamma undecalactone is present. Let mixt. stand 15–20 min., place on steam bath, and heat until ammoniacal odor is no longer evident. Add 1 ml *n-butyl alcohol* and warm until soln is clear, adding few addnl drops of the alcohol if necessary to dissolve residue completely. Remove from steam bath and let butyl alcohol evap. spontaneously. (This usually occurs overnight, but longer time may be necessary if much butyl alcohol has been used. Examine colorless or slightly yellowish crystals under microscope. If optical properties of crystals, **9.078**, correspond to those of hydrazino-γ-undecalactone, presence of γ-undecalactone is indicated. (Hydrazino-γ-undecalactone has characteristic odor similar to that of the lactone itself.)

9.078 Optical-Crystallographic Properties of Hydrazino-γ-Undecalactone —Official

In ordinary light substance appears as lath-like rods, many more or less split at ends. In parallel polarized light (crossed nicols), substance is characterized by not extinguishing sharply, most rods remaining essentially bright when stage is rotated. Occasionally crystals are found that extinguish sharply, have square ends, and show straight extinction and negative elongation. In convergent polarized light (crossed nicols) partial biaxial interference figures, usually showing one optic axis up or slightly inclined to normal, are frequent. Refractive indices, as detd by immersion method, are: $\alpha = 1.483$ (not common); $\beta = 1.525$ (most frequent and shown lengthwise on rods); $\gamma = 1.555$ (occurring crosswise on rods which show straight extinction and negative elongation); all ± 0.003. *See also* **32.234–32.236**.

Benzaldehyde (*11*)—Official

9.079 REAGENT

Phenylhydrazine soln.—Add 1.5 ml HOAc and 1 ml freshly distd phenylhydrazine to 20 ml H_2O and filter thru moistened, double S&S No. 589 white ribbon paper.

9.080 DETERMINATION

Measure sample contg 30 ml absolute alcohol into distg flask, dil. to such vol. that mixt. will contain 300 ml H_2O in addn to that required to dissolve sugar present (1 g sugar requires 0.5 ml H_2O), and distill 300 ml into 500 ml erlenmeyer. Add 10 ml phenylhydrazine soln and shake 5 min. Filter on gooch with thin mat, and wash with H_2O and finally with two 10 ml portions 10% alcohol. Dry 24 hr in vac. desiccator over H_2SO_4, excluding light, or 2 hr at 70° under 100 mm or less pressure. Wt ppt × 0.5408 = wt benzaldehyde.

9.081 Thujone (*12*)—First Action

To 500 ml sample add 1 ml *freshly distd aniline* and 1 ml H_3PO_4, and reflux 30 min. on steam bath. Distill two 100 ml portions; reject first and test second for thujone as follows:

Add 0.5 g *semicarbazide hydrochloride* and 0.6 g anhyd. NaOAc (or 1.0 g crystd salt) and let mixt. stand overnight. Distill off alcohol at as low pressure as possible. Steam distill to remove essential oils and other volatile material; collect and reject first ca 15 ml distillate. Wash down condenser with little alcohol and with H_2O. Cool sample, add 1 ml H_2SO_4 (1+1), and again steam distill, collecting 20 ml distillate in cylinder. Pour distillate into small separator, and add 20 ml ether, using receiver as the measure. Shake and sep. ether soln. Add 10 ml 65% alcohol and let ether evap. spontaneously. After all ether evaps, note odor of residue. Odor of thujone will be apparent if 2 mg or more is present in soln, provided it is not masked by presence of other odoriferous substances. Make modified Legal test as follows:

To soln obtained as above, add 1 ml *10% $ZnSO_4$ soln* and 0.25 ml freshly prepd *aq. Na nitroprusside soln* (0.1 g/ml). Slowly, with constant stirring, add 2 ml 5% NaOH soln. Let stand 1–2 min. Add 1.5 ml HOAc and mix. Ppt of raspberry red color (resembling alcohol ppt of red fruit juice) shows presence of thujone. Negative test is shown by similar ppt having appearance similar to that of alcohol ppt from apple jelly or other light colored fruit.

SELECTED REFERENCES

(*1*) J. Assoc. Offic. Agr. Chemists **38,** 822 (1955).
(*2*) Ibid. **40,** 436(1957); **42,** 327(1959).
(*3*) Ind. Eng. Chem. **18,** 841(1926).
(*4*) J. Assoc. Offic. Agr. Chemists **37,** 921 (1954).
(*5*) Ibid. **42,** 331(1959).
(*6*) Ibid. **37,** 861(1954); **43,** 657(1960).
(*7*) Ibid. **41,** 121(1958).
(*8*) J. Am. Chem. Soc. **27,** 964(1905); Ind. Eng. Chem. **19,** 844(1927).
(*9*) J. Assoc. Offic. Agr. Chemists **35,** 255 (1952).
(*10*) Ibid. **16,** 420(1933); **19,** 75, 183(1936).
(*11*) Ibid. **19,** 182(1936); **24,** 63(1941).
(*12*) Ibid. **19,** 120(1936); **20,** 69(1937); Schweiz. Wochschr. **49,** 337, 507(1911); Ann. chim. anal. **13,** 227(1908).

10. Beverages: Malt Beverages, Sirups and Extracts, and Brewing Materials*

BEER

(Unless otherwise directed, express results as % by wt)

10.001 Preparation of Sample—Official

Remove CO_2 by transferring sample to large flask and shaking, gently at first and then vigorously, keeping temp. of beer at 20–25°. If necessary, remove suspended material by passing the CO_2-free beer thru dry filter paper.

Color—Official

10.002 *Tintometer Method*

Proceed as in **10.074(a)**, dilg sample if necessary to obtain reading of 1–5°. Filter or centrifuge beers showing opacity.

Dye Method(1)†

10.003 REAGENTS

(a) *Mercuric chloride soln.*—Satd (ca 80 g/L).

(b) *Stock dye soln.*—Dissolve 0.2636 g amaranth (FD&C Red No. 2, Colour Index No. 16185), 0.6948 g tartrazine (FD&C Yellow No. 5; Colour Index No. 19140), and 0.0487 g patent blue V. F. extra conc., in 200 ml H_2O. Add 200 ml MeOH and dil. to 1 L in vol. flask. Transfer to amber g-s. bottle and store in dark. (Under these conditions soln is stable indefinitely.)

(c) *Color reference solns.*—Prep. by one of following methods:

(1) Add, from buret, vol. of stock soln in ml corresponding to dye color value (Lovibond equiv.) desired. For range 1–5° in 0.25° intervals, use 1 ml, 1.25 ml, 1.5 ml, etc., each dild to 500 ml in vol. flask. Before final vol. is reached, add 10 ml of the $HgCl_2$ soln to each flask as preservative. Mix thoroly, pour these solns into properly labeled 4 oz comparison bottles (Owens-Illinois No. A-4906 is suitable), and keep in closed box or covered comparison rack.

(2) Add, from buret, 12 ml stock soln to 1 L vol. flask and dil. to vol. with H_2O. Further dil. this 6° stock soln to make lower values as follows:

Color reference soln, degrees	1	1¼	1½	1¾	2	2¼
Ml 6° stock soln/120 ml	20	25	30	35	40	45
Color reference soln, degrees	2½	2¾	3	3¼	3½	3¾
Ml 6° stock soln/120 ml	50	55	60	65	70	75
Color reference soln, degrees	4	4¼	4½	4¾	5	
Ml 6° stock soln/120 ml	80	85	90	95	100	

(First 4 values are not usually required for beer. Therefore, for beer color sets, prep. colors from 2 to 5°, instead of 1 to 5°.)

Add, from buret, these indicated vols 6° stock soln to labeled 4 oz comparison bottles. Add 5 ml of the $HgCl_2$ soln to each bottle and dil. to 120 ml by adding H_2O in appropriate vol. from buret. (For example, the 3° color reference soln contains 60 ml 6° stock soln, 5 ml $HgCl_2$ soln, and 55 ml H_2O.) Keep finished color reference solns in closed box.

10.004 DETERMINATION

Pour prepd sample, **10.001,** into comparison bottle and match with color reference solns, **10.003(c),** against source of constant, diffused light (e.g., white blotter illuminated by frosted light bulb or fluorescent light). Match by placing sample between consecutive color reference soln bottles and viewing thru longer axis of bottle, repeating until proper pair is found. (It is often possible to interpolate value between consecutive reference solns.) Report in terms of dye color degrees, which are equiv. to the Lovibond degrees obtained by using ½″ cell and Series 52 glasses.

* Many methods in this chapter have been tested by both American Society of Brewing Chemists and Association of Official Agricultural Chemists and have been adopted by both associations. See "Methods of Analysis, A.S.B.C.," 6th rev. ed., 1958.

† Complete app., stock soln, bottles, and dyes can be obtained from Fisher Scientific Co., 2850 S. Jefferson Ave., St. Louis 18, Mo.

Spectrophotometric Method (Standard Reference Color Method) (2)

10.005 APPARATUS

Spectrophotometer.—Capable of isolating band width of 1 mμ or less at 430 mμ with wavelength and photometer scales checked and corrected for inaccuracies in accordance with instructions contained in NBS Letter Circular LC-1017 of Jan. 1955.

10.006 PREPARATION OF SAMPLE

Partially degas sample by opening bottle at room temp., pouring contents into 1 L erlenmeyer, and swirling gently. Avoid formation of turbidity, and conduct partial degassing and readings as rapidly as possible.

10.007 DETERMINATION

Place prepd sample in suitable cell and det. absorbance at 430 mμ and at 700 mμ.

Calc. absorbances from thickness at which read to ½" ($A_{1/2}$). If $(A_{1/2 \text{ at } 430 m\mu}) \times 0.039 > (A_{1/2 \text{ at } 700 m\mu})$, sample is assumed "free of turbidity" and color is calcd as follows:

Beer color intensity $= 10(A_{1/2 \text{ at } 430 m\mu})$. If $(A_{1/2 \text{ at } 700 m\mu}) > 0.039 \times (A_{1/2 \text{ at } 430 m\mu})$, use following formula:

$$\text{Beer color intensity} = 10\{(A_{1/2 \text{ at } 430 m\mu}) - [(A_{1/2 \text{ at } 700 m\mu}) - 0.039(A_{1/2 \text{ at } 430 m\mu})]\}.$$

Report color intensity values to nearest 0.1 unit.

Photometric Method

10.008 APPARATUS

Use any commercially available filter photometer or abridged spectrophotometer utilizing moderately broad spectral band and having adequate sensitivity. Use light filter with peak transmittance in range 420–450 mμ (blue-violet) for max. sensitivity and precision. (Filters for wavelengths in blue or blue-green range may also be used, but result in reduced precision.) Cell should be of such size, if possible, as to give absorbance values between 0.187 and 0.699 (20–65% transmittance), where max. precision is achieved. Use same size cell for both color measurement and calibration.

10.009 CALIBRATION OF PHOTOMETERS

Beer calibration method.—For each color intensity value for which measurements are to be made, obtain 6–8 replicate bottles of beer which are low in air content and which have been pasteurized.

Det. color intensity value of the beer by averaging readings obtained for at least 2 bottles by *Standard Reference Color (SRC) Method,* **10.007**. If these values must be obtained from another laboratory, ship bottles of beer by the fastest available method, marked to avoid rough handling.

Det. photometer reading of the beer by averaging readings obtained for at least 2 bottles with wavelength and cell as in **10.008**. Calc. calibration factor in accordance with photometer instructions or prep. calibration curve by plotting absorbance or photometer scale reading against the *SRC* value for sample, assuming that curve passes thru origin. This calibration will be accurate only for readings in immediate vicinity of calibration point. If it is desired to measure accurately color intensity of more than one sample or colors over range of values, calibrate photometer for each sample or use beers having colors which cover desired range. Calc. av. calibration factor or prep. av. calibration curve.

10.010 PREPARATION OF SAMPLE—*See* **10.006**

10.011 DETERMINATION

Place sample in cell and det. photometer reading. Calc. color intensity value, using calibration factor or calibration curve. Report color to nearest 0.1 unit.

Turbidity—First Action

Formazin Methods (3)

10.012 REAGENTS

(Use turbidity-free distd H_2O thruout.)

(**a**) *Hydrazine sulfate soln.*—1%. Dissolve 1.000 g hydrazine sulfate in H_2O (may require 4–6 hr) and dil. to 100 ml.

(**b**) *Stock formazin suspension.*—Dissolve 2.500 g hexamethylenetetramine (formin) in 25 ml H_2O in 125 ml erlenmeyer, pipet in 25 ml hydrazine sulfate soln, (**a**), and stopper flask. Formazin begins to ppt in 6–8 hr and pptn is complete within 24 hr. Prep. every 3 months.

(**c**) *10,000 Turbidity std.*—Dil. 14.5 ml well-mixed stock suspension, (**b**), to 100 ml with H_2O in vol. flask. Prep. weekly. (10,000 formazin turbidity units (FTU) on empirical formazin turbidity scale represents reflectance of insol. reaction products of 0.0725 g hydrazine sulfate with 0.7250 g hexamethylenetetramine dild to 100 ml.)

(**d**) *1000 Turbidity std.*—Dil. 10 ml well-mixed 10,000 turbidity standard, (**c**), to 100 ml with H_2O in vol. flask. Prep. weekly.

(**e**) *Working stds for visual method.*—Prep. following dilns of the 1000 turbidity std in 100 ml comparator tubes (increase vols proportionally if larger tubes are used):

Turbidity value (FTU)	ml 1000 turbidity std	ml H_2O
0	0	100
20	2	98
40	4	96
60	6	94
100	10	90
140	14	86
180	18	82
220	22	78
260	26	74
300	30	70

The 0, 20, 40, and 60 FTU stds cover range generally encountered with finished or freshly bottled samples; higher stds may be required for samples with bottle age.

10.013 PREPARATION OF SAMPLE

Chill bottle sample 24 hr at desired temp. Stir gently with glass rod. If ppt has settled on bottom, brush free with rubber policeman, and disperse by stirring. (If effect of sediment is not desired, omit removal of ppt and report as "without sediment.")

Visual Methods

10.014 APPARATUS

Clark turbidimeter.—Model 756. Available from Cargill Scientific Co., 118 Liberty St., New York 6, N.Y. Viewing box of same dimensions and lighting is also suitable.

10.015 DETERMINATION

Chill comparator tube by holding it in ice-H_2O bath contg few drops wetting agent. Rinse tube with sample and refill immediately (degassing is not necessary). Dip tube in ice bath to remove any spilled beer. Place sample tube in turbidimeter between 2 consecutive working stds and view thru piece of red or orange glass or cellophane to nullify color differences. In range 0–60 units, report turbidity to nearest 10 unit; 60–180, 20; >180, 40. Specify testing temp.

Nephelometric Method

10.016 APPARATUS

Nephelometer.—With matched cuvettes. When using nephelometer adapters supplied with Coleman models 11 or 14, waterproof joints between photocell glass cover and Bakelite sample holder by applying thin layer of clear lacquer to exterior periphery. Use wavelength of 580 mμ and set blank at 0% reflectance with H_2O and at 100% with 1000 turbidity std. (If sensitivity of instrument is too low to permit this adjustment, prep. and use 2000 FTU std.) Recheck blank and std settings occasionally during series of tests.

10.017 DETERMINATION

Chill cuvette by holding it for several sec. in ice-H_2O bath contg few drops wetting agent. Do not get any of this soln in cuvette. (Wetting agent may be omitted with instruments contg H_2O basket sample chamber.) Rinse cuvette with sample and refill immediately. Immerse in ice bath and stir with thermometer of 30–70°F range to degas. Keep sample at desired temp. by holding cuvette in or out of cold bath as required. Stir until all bubbles have been removed, ca 15 sec. If nephelometer does not have H_2O basket sample chamber, remove cuvette from cold bath and quickly blot excess H_2O from corners, edges, and bottom with absorbent paper. Do not touch area thru which light will pass. Place cuvette in sample chamber and read % reflectance to nearest 0.5% from galvanometer scale. Remove cuvette, and with instruments that do not have H_2O basket sample chamber, dry sample chamber immediately with absorbent paper, if necessary.

If 1000 turbidity std was used for stdzn, turbidity value = % reflectance × 10; if 2000 std was used, turbidity value = % reflectance × 20. Report turbidity to nearest 5 units. Specify testing temp.

10.018 Specific Gravity—Official

Det. sp. gr. of prepd sample, **10.001**, at 20/20° (in air) as in **9.011**.

10.019 Apparent Extract—Official

Find apparent ext. corresponding to sp. gr. detd at 20/20° from **43.003**, reporting to second decimal place.

10.020 Alcohol—Official

(**a**) *By volume.—See* **11.004**.
(**b**) *By weight.—See* **9.021**.

10.021 Real Extract—Official

(**a**) Evap. 75–100 ml sample (accurately weighed to 0.1 g) on H_2O bath or asbestos plate, at temp. not >80°, to ca ⅓ original vol. Cool, make to original wt with H_2O, and det. sp. gr. with pycnometer at 20/20°. Det. real ext. directly from **43.003.**

(**b**) If no anti-foam material was used in detn of alcohol, **10.020**, transfer residue quantitatively with hot H_2O to 100 ml vol. flask. Cool, and dil. to 100 ml at 20°. Det. sp. gr. at 20/20°, **10.018**, and find ext. directly from **43.003.** If 100 ml beer was taken, correct as follows:

Ext. found × sp. gr. of dealcoholized beer /sp. gr. of beer = g ext./100 g beer.

10.022 Extract of Original Wort—Official

Calc. from following formula and report to first decimal place:

$$O = \frac{(A \times 2.0665) + E}{100 + (A \times 1.0665)} \times 100, \text{ in which}$$

O = ext. of orig. wort; A = % alcohol by wt (g/100 g beer); and E = % real ext., **10.021(a)** or (**b**).

10.023 Real Degree of Fermentation or Real Attenuation—Official

Calc. as follows and report to first decimal place:

(orig. ext. − real ext.) × 100/orig. ext.

10.024 Apparent Degree of Fermentation or Apparent Attenuation—Official

Calc. as follows and report to first decimal place:

(orig. ext. − apparent ext.) × 100/orig. ext.

Total Acidity (*4*)—Official

10.025 *Indicator Titration Method*

Bring 250 ml H_2O to boil and continue boiling 2 min. From fast flowing pipet add 25 ml beer previously decarbonated by shaking and filtering, **10.001.** After emptying pipet, continue heating 60 sec., regulating heat so that soln resumes boiling during final 30 sec. Remove from heat, stir 5 sec., and cool rapidly to room temp.

Add 0.5 ml 0.5% phthln. Titr. with 0.1*N* NaOH against white background. Make frequent color comparisons with sample of equal vol. and diln to which has been added approx. anticipated quantity of alkali but no indicator. Titr. to first appearance of faint pink. Read buret. Add 0.2 ml more alkali; color should then be permanent, definite pinkish-red, indicative of over-titrn. Take first buret reading as end point.

Observe strictly all details of method. However, 100 ml H_2O, 10 ml beer, and 0.2 ml indicator may be used in place of quantities specified above. (Use potentiometric titrn method, **10.026,** for beers of dark color which (even when dild) may not permit judging phthln end point with necessary precision.)

Report results: (**a**) as lactic acid, to nearest 0.01% (1 ml 0.1*N* alkali = 0.0090 g lactic acid); or (**b**) as ml 1*N* alkali, to nearest 0.1 ml, necessary for neutralization of 100 g beer.

10.026 *Potentiometric Titration Method*

Use glass-calomel electrode system. Decarbonate beer completely by shaking, **10.001.** Using 50 ml undild sample (or such quantity as best suits titrn assembly), titr. potentiometrically with 0.1*N* NaOH to pH 8.2. Add alkali in 1.5 ml portions to ca pH 7.6, and in 0.15 ml portions from there to pH 8.2. Make sure that complete equilibrium and good convergence are attained before reading buret at exactly pH 8.2. Report results as in **10.025.**

PRECAUTIONS: Observe all details of good potentiometric technic, including following: Stdze potentiometer against fresh 0.05*M* K acid phthalate, **42.007(c)**, before and after any set of titrns; read potentiometer to nearest 0.02 unit; use flexible shielding around electrode leads and motor cords; ground motor and motor cords, preferably to H_2O pipes; avoid contact between electrodes and glass beaker; use proper stirring speed to assure quick mixing but to avoid foaming which may temporarily trap some of alkali added; stop titrn at not over pH 8.6 to minimize alkali contamination of glass electrode; check batteries frequently. Follow instructions issued by manufacturer of potentiometer used.

10.027 Hydrogen-Ion Concentration (pH)—Official

Electrometric Method

Det. pH of undild sample, **10.001,** using glass-calomel electrode system. Follow instructions issued by manufacturer of potentiometer used. Check pH meter before and after use against std K acid phthalate buffer, **42.007(c)**. Observe precautions in **10.026.** Report results to nearest 0.05 pH.

10.028 Volatile Acids—Official

Using 100 ml beer, proceed as in **11.030(b)**. Express result as HOAc, g/100 ml. 1 ml 0.1*N* alkali = 0.0060 g HOAc.

10.029 Reducing Sugars—Official

Dil. 25 ml prepd sample, **10.001,** measured at 20°, to 100 ml with H_2O at same temp. Det. reducing sugars in 25 ml of this soln by Munson-Walker method, **29.065,** or dil. 50 ml beer with H_2O to 100 ml and use Lane-Eynon method, **29.064.** Express result as g maltose/100 ml beer. For conversion to % by wt, divide results by sp. gr. of beer.

10.030 Dextrin—Official

To 25 ml prepd sample, **10.001,** measured at 20° in erlenmeyer, add 15 ml HCl (sp. gr. 1.125) and dil. to 200 ml. Attach flask to reflux condenser, and keep in boiling H_2O bath 2 hr. Cool, nearly neutralize with NaOH soln (1+1), dil. to vol. of 250 ml, filter, and det. dextrose as in **29.054.** [Dextrose, g/100 ml − (1.053 × maltose, **10.029**)] × 0.9 = g dextrin/100 ml beer.

10.031 Glycerol—Official—*See* 11.012(b)

10.032 Ash—Official

Evap. to dryness 50 ml prepd sample, **10.001,** measured at 20°. Proceed as in **29.012** or **29.013.**

10.033 Phosphorus—Official

To 50 ml prepd sample, **10.001**, measured at 20°, add 20 ml *2% $Ca(OAc)_2$ soln*, evap. to dryness, and ignite at low redness to white ash. Add 10–15 ml boiling HNO_3 (1+9) and det. P_2O_5 as in **2.022.** (Washing phosphomolybdate ppt with 1% KNO_3 soln instead of H_2O prevents creeping.)

10.034 Protein—Official

To 25 ml prepd sample, **10.001**, at 20° in Kjeldahl flask, add 2–3 ml H_2SO_4 and conc. to sirupy consistency. Det N as in **2.036.** % N×6.25 = % protein.

$$\% \text{ protein} = [(\text{ml } 0.1N \text{ acid} - \text{ml } 0.1N \text{ base}) \times 1.4 \times 6.25 \times 100]/(\text{sp. gr.} \times \text{ml sample} \times 1000).$$

Carbon Dioxide—Official

Manometric Method (5)

10.035 APPARATUS

(a) *Piercing apparatus.*—(1) *For bottles.*—Consists of gas-tight packing box and fastening for adjustment over container, and hollow spike connected to accurate pressure gauge and outlet valve. Check gauges frequently. (2) *For cans.*—Consists of metal frame in which can is placed. Top of app., which is pressed or screwed down and locked over can top, contains hollow spike surrounded by compressible rubber sealing plug; hollow spike leads to accurate pressure gauge and outlet valve. (One app., adjustable for use with both bottles and cans, may be employed.)

NOTES: Piercing devices can be obtained from Liquid Carbonic Corp., 155 E. 44th St., New York 17, N.Y.; Zahm and Nagel Co., Inc., 74 Jewett Ave., Buffalo 14, N. Y.; and Micromat Co., 548 Piermont Ave., Hillsdale, N.J.

For suitable manometer for calibrating gauges, *see* Gray and Stone, *Ind. Eng. Chem., Anal. Ed.*, **10,** 15 (1938). Dead wt testing unit suitable for calibration can be obtained from Mansfield and Green, 1051 Power Ave., Cleveland, Ohio; Amthor Testing Instrument Co., Inc., 45 Van Sinderen Ave., Brooklyn 7, N. Y.; Ashcroft Gauge Division, Manning, Maxwell and Moore, Inc., 250 E. Main St., Stratford, Conn., and other companies.

(b) *Absorption buret.*—(Fig. 20). Consists of graduated tube (one type has 0–5 ml graduated in 0.05 ml divisions, 5–15 ml in 0.1 ml, and 15–25 ml in 0.5 ml) with bulb marked at 40 ml, and closed at each end by stopcocks. Connect buret to valve of piercing app. and to leveling bulb by transparent alk.-resistant plastic or rubber tubing. (Burets are available from Zahm and Nagel Co. and Micromat Co., (a), and from N. Y. Laboratory Supply Co., 76 Varick St., New York 13, N.Y.)

(c) *Leveling bulb.*—Approx. 300 ml, with support.

10.036 DETERMINATION

Bring samples to 25° by immersion in H_2O bath at 25°. If sample is bottle, make scratch on bottle at beer level. If sample is can, weigh unopened can.

Fill leveling bulb and then absorption buret with 15% NaOH soln. Displace air in tubing connecting it to piercing app. completely with H_2O or NaOH soln and attach piercing device to bottle or can. Take care that no air is trapped in system that will be carried into buret during detn.

With valve of piercing device closed, pierce bottle crown or can by depressing hollow steel spike. Shake bottle or can until pressure reaches constant max. value. Stop shaking and record pressure reading. Open valve on piercing app. cautiously and let gas-foam mixt. flow into absorption buret until pressure gauge reads zero. Close valve and shake or tip buret (depending on its construction) until CO_2 is absorbed and gas vol. in buret reaches max. value. Adjust leveling bottle to equalize hydrostatic pressure and read vol. of "headspace air" contained in buret.

If detn of "total air" is also desired, continue evolution of gas from bottle or can by shaking it. Absorb evolved CO_2 by swirling and shaking buret. Continue shaking and CO_2 absorption until there is no further increase in vol. of unabsorbed gas in buret. Final vol. of unabsorbed gas may be considered the "air content" or "total air" of container.

Disconnect piercing device from package and insert thermometer to be sure that temp. is 25°. Det. headspace vol. as follows:

(a) *Bottles.*—Fill bottle to top with H_2O and pour from it into 100 ml graduated cylinder until liquid level in bottle corresponds to scratch mark placed on it. Vol. in ml of liquid poured off is headspace vol.

(b) *Cans.*—Empty beer from can and let it drain completely. Weigh empty can. Fill empty can with H_2O and weigh. Subtract wt of empty can from wt of unopened can of beer to obtain wt of beer before opening the can. Divide beer wt by sp. gr. of the beer to obtain vol. of beer in can in ml. Subtract wt of empty can from wt of can filled with H_2O. Difference is wt of H_2O, equiv. to vol. in ml required to fill can completely. Subtract vol. of beer from vol. of can to obtain headspace in can before opening. (This detn of headspace in cans is only approximately correct, due to unknown degree of bulging of cans under pressure, distortion of end on opening or puncturing can, and difficulty of accurately defining when can is completely filled with H_2O.)

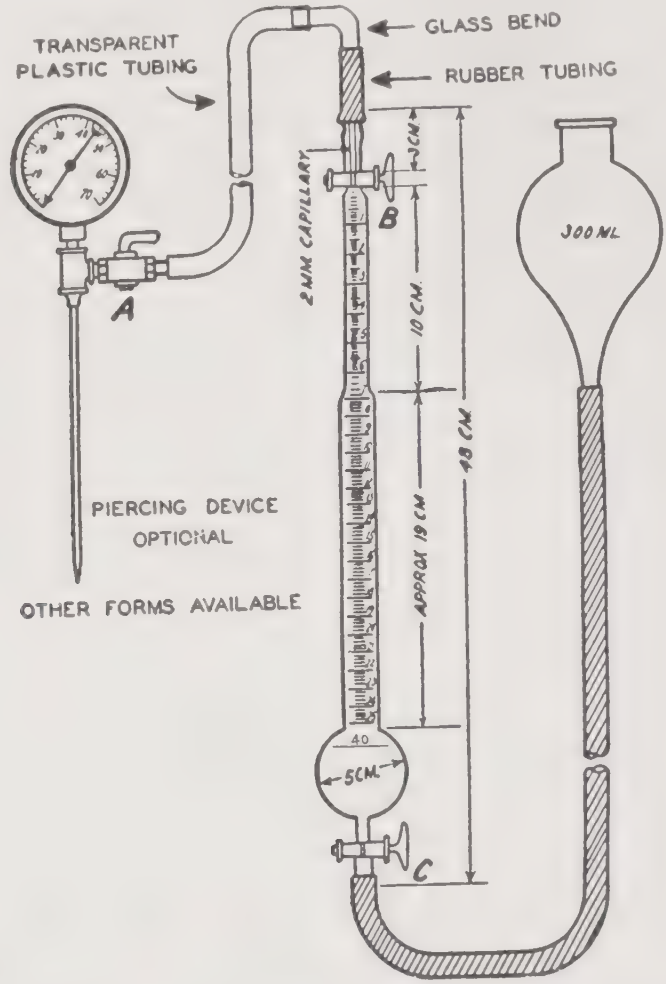

FIG. 20.—ABSORPTION BURET (OTHER FORMS AVAILABLE)

Calc. % CO_2 by wt and vol. by following formulas:

% CO_2 by wt $= [P - (\text{ml “head-space air”} \times 14.7/\text{ml headspace})] \times 0.00965$, where P = absolute pressure in lbs/sq. in. = gauge pressure + 14.7.

Vols CO_2 = % CO_2 by wt × sp. gr. of beer/0.1976 = % CO_2 by wt × 5.0607 × sp. gr. of beer.

Report % by wt to second decimal, and vols CO_2 to first decimal.

10.037 Sulfur Dioxide—Official

Proceed as in **27.078**, except to add 300 ml beverage (not decarbonated) thru dropping funnel, with no addnl H_2O, followed by 20 ml HCl. Let mixt. stand few min. until fumes settle. Adjust burner so that vapors rise no higher than 1/10 length of H_2O jacket of condenser and boil 90 min. Adjust flow of CO_2 so that slow but steady stream passes thru receiver during distn, and complete detn as in **27.078**. Report results as mg SO_2/L.

10.038 Iodine Reaction for Unconverted Starch—Procedure

(**a**) *For light beer.*—Fill 15 mm diam. test tube to within 1″ from rim with beer, **10.001**. Carefully add 0.02N I from dropper to form distinct layer on top of beer. Observe at once, by transmitted light, color developed at interface. Report blue color as indicating presence of starch; purple color, amylo-dextrin; and reddish color, erythrodextrin. Qualify results by using terms faint trace, trace, and plain trace according to whether the color developed is faint, distinct, or strong.

(**b**) *For dark beer, but applicable also to a light beer.*—To 5 ml beer in test tube add 25 ml alcohol, shake thoroly, and let stand. Decant, pouring off last trace of beer-alcohol mixt. Dissolve ppt (dextrin) in 5 ml H_2O and to this soln add 0.02N I soln dropwise. Interpret as in (**a**).

Copper

Direct, Non-ashing Method (6)—First Action

10.039 REAGENTS

(**a**) *Zinc dibenzyldithiocarbamate (ZDBT) soln.*—0.5%. Dissolve 5 g ZDBT (available from Naugatuck Chemical Co., Naugatuck, Conn., under trade name “Arazate”) in toluene and dil. to 1 L with toluene. Filter, if necessary, thru Whatman No. 42 paper, and store in brown

bottle in cool, dark place. CCl_4 may be used instead of toluene.

(**b**) *Copper std soln.*—Dissolve 3.93 g $CuSO_4 .5H_2O$ (free of any whitish deposit of lower hydrates) and dil. to 1 L with H_2O (1 ml = 1 mg Cu). Or dissolve 1.000 g pure Cu wire or foil in 75 ml HNO_3 (1+4) by warming, boil to expel fumes, cool, and dil. to 1 L with H_2O. Prep. dil. std soln (1 ml = 0.01 mg Cu) immediately before use by dilg 5 ml of the std soln with Cu-free distd H_2O to 500 ml in vol. flask.

(**c**) *Copper-free distilled water.*—Shake out distd H_2O with ZDBT soln in separator.

10.040 APPARATUS

(**a**) *Photometer.*—Any commercial instrument with blue filter (430–460 mμ) or spectrophotometer set at 435 mμ.

(**b**) *Copper-free centrifuge tubes.*—Clean and rinse 50 ml centrifuge tubes; add 15 ml H_2O, 3 ml H_2SO_4 (1+3), and 5 ml of the ZDBT soln. Stopper with corks or glass stoppers and shake thoroly. Discard soln and let tube drain.

10.041 PREPARATION OF STANDARD CURVE

Into series of cleaned, corked or g-s. 50 ml centrifuge tubes add 0.0, 1.0, 2.0, 3.0, 4.0, and 5.0 ml of the dil. Cu std soln, equiv. to 0.0, 0.4, 0.8, 1.2, 1.6, and 2.0 ppm Cu, resp. Add 25 ml beer, degassed as in prepn of sample, **10.042,** and 1 drop *hexyl alcohol*; mix, and proceed as in detn, **10.043.**

Color over this range follows Beer's law. Calc. factor to convert absorbance to ppm Cu after subtracting absorbance of std contg 0.0 ppm Cu from those contg added Cu. If instrument response is not linear, use calibration curve.

10.042 PREPARATION OF SAMPLE

Cool bottle or can and shake thoroly immediately before opening. Let gas bubbles leave liquid before removing cap or puncturing can. Discard ca 1/3 of sample and degas by swirling. Remove sample directly from container.

10.043 DETERMINATION

To cleaned 50 ml centrifuge tube add 25 ml cold sample, measured in graduated cylinder, 3 ml H_2SO_4 (1+3), and 1 ml 30% H_2O_2. If foam interferes with sample measurement, add 1 drop hexyl alcohol. Mix, and place tube in boiling H_2O bath 0.5 hr. If excessive foaming occurs, add 1 drop hexyl alcohol. Remove tube and cool to 25°. Add 5 or 10 ml, accurately measured, of the ZDBT soln, depending upon size of photometer cell, and stopper tube. Ext. at 25° by shaking vigorously 60 times. Re-ext. again 4 times, giving 60 snapping shakes each time to obtain fine emulsion, allowing partial sepn between extns. Digested sample must be shaken vigorously with the ZDBT soln; thoro and complete emulsification must be obtained during each series of extns or results may be low.

Centrifuge tube 2–3 min. and draw off clear, colored layer to photometer cell of same size used in calibration, and det. absorbance, A_{samp}. If droplets of aq. layer are carried into pipet, remove by flowing solvent from pipet down wall of clean, dry test tube. H_2O droplets will adhere to test tube and clear solvent can be poured off into cell.

Prep. reagent blank by extg in clean 50 ml centrifuge tube 25 ml Cu-free H_2O at 25° and 3 ml H_2SO_4 (1+3) with 5 (or 10) ml ZDBT soln and det. absorbance, A_1. To correct for absorbance of color extd by solvent, perform entire detn, omitting ZDBT soln, but shaking with toluene (or CCl_4), and det. absorbance, A_2. Do not give tubes used for this solvent-extractable beer color blank preliminary cleaning with ZDBT soln, since carryover of ZDBT may give high readings.

Ppm Cu $= [A_{samp} - (A_1 + A_2)] \times f$, where f is factor for converting absorbance to ppm Cu.

Iron (*7*)—Official

10.044 APPARATUS

Photometer.—Spectrophotometer set at ca 505 mμ or photometer equipped with filter in blue-green region, 500–550 mμ, or preferably, 505–520 mμ.

10.045 REAGENTS

(**a**) *Color reagent:* (1) *2,2′-bipyridine.*—0.2%. Dissolve 1 g 2,2′-bipyridine in 20 ml HOAc (1+2) and dil. to 500 ml with H_2O; or—

(2) *o-Phenanthroline.*—0.3%. Dissolve 1.5 g *o*-phenanthroline in 500 ml H_2O at 70°.

(**b**) *Iron std soln.*—1 ml = 0.1 mg Fe. (1) *From iron wire.*—Dissolve 0.500 g reagent grade Fe wire, wiped free of oxide, in 5 ml HCl (1+4) and 1 ml HNO_3. Cover with watch glass, heat, and evap. to dryness; add H_2O and evap. to dryness again. Dissolve residue in 3–5 ml HCl, cool, and transfer quantitatively to 500 ml vol. flask. Add 2 drops satd Br-H_2O, dil. to mark with H_2O, and mix. Transfer 50 ml of this soln to 500 ml vol. flask, add 2 drops Br-H_2O, dil. to mark with H_2O, and mix.

(2) *From ferrous ammonium sulfate.*—Dissolve 3.512 g $Fe(NH_4)_2(SO_4)_2.6H_2O$ in H_2O, add 5 ml HCl, transfer quantitatively to 500 ml vol. flask, dil. to mark with H_2O, and mix. Transfer 50 ml of this soln to 500 ml vol. flask, dil. to mark with H_2O, and mix.

(**c**) *Ascorbic acid.*—USP, ground to fine powder.

10.046 PREPARATION OF STANDARD CURVE

Prep. series of *beer stds* contg 0.0, 0.25, 0.50, 1.00, 2.00, and 3.00 ppm Fe as follows: Pipet 0.0, 0.25, 0.50, 1.00, 2.00, and 3.00 ml std Fe soln, **10.045(b)**, to series of 100 ml vol. flasks, add,

from pipet, enough H_2O to total 3.00 ml, and dil. to mark with decarbonated beer, **10.047**. Depending upon size cell to be used, develop color in 25 or 50 ml aliquots of each of the beer stds as in **10.048**(a) or (**b**).

If transmittance values, T, are obtained, convert to absorbance, $A = -\log T$, and plot A against ppm Fe. If straight line results, calc. factor, m, for converting A to ppm Fe, y, by use of equation $y = mA + b$ ($b = 0$ if line passes thru origin). If instrument response is such that curve is obtained, use this curve to calc. results.

10.047 PREPARATION OF SAMPLE

Adjust temp. of beer to 20–25°. Decarbonate by transferring sample to large erlenmeyer and shaking, first gently and then vigorously, until all gas is released. Do not filter unless necessary. If filtration is required, make sure filter paper is Fe-free by spotting sample of the paper with drop of reagent prepd by dissolving 25 mg ascorbic acid in 2 ml color reagent, **10.045(a)**.

If beer sample is suspected of high Fe content, degas by shaking only, and permit foam to subside before sampling.

10.048 DETERMINATION

Pipet 2 aliquots of degassed beer (25 or 50 ml as used in prepn of std curve) into 50 ml or 125 ml erlenmeyers; add 25 mg ascorbic acid to each aliquot, and 2 ml of color reagent, **10.045**(a), to one and 2 ml H_2O to other. (**a**) Stopper and heat both aliquots 15 min. at 60°, or (**b**) let stand 30 min. at room temp.

Cool, and read both solns in photometer against H_2O as reference, or read the colored aliquot against beer blank as reference. Use same size cell and wavelength as used in prepn of std curve.

10.049 CALCULATIONS

If H_2O is used as reference and factor is used, ppm $Fe = (A_{sample} - A_{blank}) \times$factor. If values are taken from std curve, ppm Fe = ppm Fe in sample − ppm Fe in blank.

If beer blank is used as reference and factor is used, ppm $Fe = A_{sample} \times$factor. If values are taken from std curve, ppm Fe = ppm Fe directly.

10.050 Other Metals—*See* Chap. 24

10.051 Chlorides—Official

Place 50 ml sample in Pt dish, add 20 ml 5% Na_2CO_3 soln, and proceed as in **6.065**. Det. Cl as in 6.066 or 6.068.

10.052 End Fermentation (Yeast Fermentable Extract) (Fermentable Sugars)—First Action

Det. real ext., **10.021**, or apparent ext., **10.019**. Ferment 250 ml beer with 1 g active compressed brewers yeast 24–48 hr at 15–25°, or until fermentation is complete, providing fermentation flask with H_2O or Hg seal. Filter; det. real ext., **10.021**, or apparent ext., **10.019**. Fermentable sugars = difference in real ext. before and after fermentation; or fermentable sugars = 0.82 × difference in apparent ext. before and after fermentation.

10.053 Caramel—Official—*See* **11.040**

MALT

10.054 Sampling—Official

For complete descriptions of trier, divider, sampler, and bushel weight tester, see "Handbook of Official Grain Standards of the United States Department of Agriculture" (1956), p. 88–95.

(**a**) *Bulk malt in cars or bins.*—Using 60″ trier, take at least 6 probes from different parts of car, preferably 2 from center and 2 from each end.

(**b**) *Bulk malt during discharge thru spouts or openings.*—At different times during filling or unloading of car, take, with trier or Pelican sampler, at least 6 samples, each representing complete cross section of grain stream from spout.

(**c**) *Bagged malt.*—Sample lengthwise thru center of open bags, not $<2\%$ of bags selected from different parts of car or storage room. Use 36″ trier.

Indicate approx. proportion of inferior grain and take representative samples from each portion as outlined above. Immediately place each portion of sample in suitable large dry container and keep tightly closed.

10.055 Preparation of Sample—Official

Divide samples, either by quartering or by using sample divider, until ca 3 lb remains. Place reduced sample in air-tight container (preferably tin with screw or friction type cover); do not use cartons, bags, wooden boxes, glass Mason jars, or wrapping paper. Remove foreign particles, such as stone, wood, and twine. Do not remove foreign seeds or dust particles.

Bushel Weight—Official

10.056 *Method I.*

Place sample in filling hopper of Winchester tester, open slide underneath, and let malt fill measuring cylinder to overflowing. Without jarring, level off with straight-edge longer than diam. of measuring cylinder, making one forward stroke consisting of 3 distinct zigzag motions. Weigh and report to nearest $\frac{1}{4}$ lb.

10.057 *Method II.* (*8*)

Weigh 110 g sample to nearest 0.1 g and pour evenly into metal funnel provided with plunger discharge and placed on top of 250 ml cylinder

graduated to meet NBS specifications. (Funnel must fit snugly into graduate and be large enough to hold the grain without danger of spilling when plunger is raised.) Then drop material into cylinder by pulling plunger up. Do not jar or tap cylinder during operation or before reading vol., and do not read uppermost grain level, as compensation must be made for ends of few kernels that protrude. If grain surface has slant, repeat test.

Calc. bushel wt of malt (lbs) as = 8545/vol. in ml of 110 g.

Constant 8545 is derived from W, the wt in lbs of US (Winchester) bushel of 2150.42 cu. in. (35,239 ml). If V = vol. in ml of 110 g malt,

$$\frac{110}{453.6W} = \frac{V}{35{,}239}; \quad W = 8545/V.$$

10.058 Length of Acrospire—Procedure

For methods (**a**) and (**b**), quarter sample until ca 200 kernels remain in 2 opposite quarters, and count out 100 kernels, rejecting those that are broken or those in which growth is not ascertainable.

(**a**) *Cutting.*—Hold each kernel furrow downward on flat surface with pair of tweezers, cut thru kernel longitudinally with razor blade or other sharp instrument, and examine cut acrospire in both halves, comparing its length with that of kernel. Tally according to classifications below.

(**b**) *Peeling.*—Remove husk covering acrospire with sharp instrument and examine acrospire length in comparison with length of kernel. Tally according to classifications below.

(**c**) *Boiling.*—Boil 10–15 g av. sample with 100–150 ml H_2O 20–30 min. After boiling, add cold H_2O to cool contents of beaker. Decant, and pour grain on glass plate. Select 100 kernels at random, inspect acrospire, and tally according to classifications below.

Classify kernels as follows and report % in each group:

- 0–¼: includes those kernels without apparent growth, or having acrospire development up to, but not including, ¼ length of grain.
- ¼–½: includes those kernels having acrospire development from ¼ up to, but not including, ½ length of grain.
- ½–¾: includes those kernels having acrospire development from ½ up to, but not including, ¾ length of grain.
- ¾–1: includes those kernels having acrospire development of ¾ but not greater than entire length of grain.
- Overgrown: includes those kernels having acrospire development in excess of length of grain.

If it is apparent that overgrown acrospire has been broken off during processing, include kernel in overgrown classification regardless of length of remaining stub.

10.059 Mealiness—Procedure

Count out 100 kernels remaining from preceding test if method **10.058**(**a**) or (**b**) was used. Otherwise select 100 kernels as in **10.058** and cut kernels in longitudinal halves. Det. % mealy, half glassy, and glassy kernels. In case of uncertainty, pierce starch body with sharp point; if mealy, it will break away and crumble from point.

Classify kernels as follows:

Mealy kernels—includes those kernels in which not >¼ of the endosperm body is glassy.

Half glassy—includes those kernels in which not <¼ nor >¾ of the endosperm body is glassy.

Glassy—includes those kernels in which ¾ or more of the entire endosperm body is glassy.

10.060 1,000 Kernel Weight—Procedure

Quarter sample until ca 500 kernels remain in 2 opposite quarters. Count out 500 kernels and weigh to nearest 0.1 g. Calc. results to 1,000 kernels on as-is and dry basis.

10.061 Assortment—Procedure

Weigh 100 g from quartered sample to nearest 0.1 g. Place in top compartment of grader* and shake 3 min. Weigh quantities remaining on various screens and in catch pan to nearest 0.1 g, and report % on each of following screens: 7/64″, 6/64″, 5/64″, and thru 5/64″, in percentages totaling 100%. (When testing large berried malts (2 row, California, etc.), addn of 8/64″ screen is optional.)

10.062 Mold—Procedure

Det. presence or absence of mold by visual inspection and report as "none," "trace," etc.

10.063 Foreign Seeds and Broken Kernels—Procedure

Weigh 50 g sample. Pick out foreign seeds and broken kernels, classify, and report separately in %.

Moisture—Official

10.064 Apparatus

(**a**) *Weighing dish.*—Use glass bottle, or Al dish with tight-fitting cover, ca 40 mm diam. for 5 g sample, or 55 mm for 10 g sample.

(**b**) *Oven.*—With automatic control capable of holding temp. within ±0.5°, and large enough to hold all samples on one shelf in such manner that no sample is outside area indicated by test to give comparable results in duplicate samples. Stdze

* Grader frame and screens are obtained from S. Howes Co., Silver Creek, N. Y.

oven as follows: Place weighed duplicate samples in oven at 103–104° and dry 3 hr. Weigh, and redry 1 hr longer. If loss of moisture is >0.1%, raise temp. 1° and again test with new duplicate samples. Take, as std, lowest temp. below 106° giving moisture content that, after 3 hr of drying, is within 0.1% of value attainable at same temp. within 4 hr. Keep ventilators of oven open during entire drying period, and do not open door during the 3 hr of drying.

10.065 PREPARATION OF SAMPLE

(**a**) *If extract determination is to be made.*—Grind sample as in **10.070,** and transfer in one continuous operation. When many samples are to be analyzed, grind first sample, remove beaker, and grind second sample while adjusting wt of first sample. Remove second sample, insert third sample, and repeat operation.

(**b**) *If extract determination is not to be made.*—Have sample of same fineness as finely ground malt used to det. ext. Weigh ca 5 g whole malt (or 10 g if 55 mm diam. weighing bottle is used) and grind thru clean dry mill directly into weighing bottle. Brush all malt from mill into weighing bottle and cover immediately.

10.066 DETERMINATION

Weigh sample to 1 mg and place in oven previously heated to std temp. Remove cover of weighing bottle and heat exactly 3 hr at std temp. Replace cover, transfer to desiccator, cool to room temp., and weigh to 1 mg. Report moisture to nearest 0.1%.

Extract—Official

10.067 REAGENT

Iodine solns.—(**a**) 0.01*N*. Dissolve 0.63 g I and 1.25 g KI in H_2O, and dil. to 500 ml. (**b**) 0.02*N*. Dissolve 1.27 g I and 2.50 g KI in H_2O, and dil. to 500 ml. Prep. fresh solns monthly.

10.068 APPARATUS

(**a**) *Mills.*—Miag-Seck. For fine grinding use cone-type, 300 rpm, and for coarse grinding roller-type, 150 rpm.

(**b**) *Sieves.*—Half-height, 8″ std sieve No. 30 (with pan and cover). For classification of laboratory and brewery grindings use addnl std sieves Nos. 10, 14, 18, 60, and 100.

(**c**) *Mash beakers and counter weights.*—Made of either pure Ni, stainless steel, or brass, not Cu, and of such dimensions as to assure tight connection between beakers and Miag-Seck mill while grinding.

If counter wts are used for the mash beakers, check tare wts frequently.

(**d**) *Mashing apparatus.*—Use beakers, stirrers, and solder made of same metal. Provide each stirrer with blade that in operation has clearance of ca 2 mm from bottom and 5 mm from wall of mash beaker. Blade is ca 8 mm wide, and each side has 45° pitch, arranged as in a propeller, to force mash upward. Speed of mash stirrer must be 80–100 rpm, each stirrer of each beaker having same speed. Stir H_2O in bath thoroly by mechanical means to assure uniformity of temp. and have level of H_2O above max. mash level.

(**e**) *Gypsum plate.*—Thoroly mix 100 ml H_2O with 135 g plaster of Paris. Pour mixt., while still free-flowing, into suitable flat molds (cigar boxes, etc.). Porcelain plate for color reactions, Coors No. 550, size 00, may be used.

(**f**) *Filter paper.*—Use S&S 32 cm fluted paper No. 560 (or No. 597, 32 cm, fluted by analyst) or Eaton-Dikeman 32 cm fluted paper No. 509 (or No. 609, 32 cm, fluted by analyst).

(**g**) *Funnels.*—Use short-stem glass funnels ca 20 cm diam. and do not let paper project above rim. Stem must extend 3–5 cm into receiving flask.

(**h**) *Flasks.*—Use dry 500 ml erlenmeyers marked at 100 ml level.

(**i**) *Pycnometers.*—Use any suitable pycnometer, but preferably Reischauer or Boot (vac.) type. Reischauer type is ca 15 cm high with neck ca 9 cm long and 2.5–3.5 mm i.d. Fine, well-defined mark is found 55–70 mm below upper rim of neck. When filled with H_2O at 20° its capacity must be 48–50 g. Use ca 15 ml capacity glass funnels to fill pycnometers.

Boot type is cylindrical and holds ca 50 g H_2O at 20°. Vac. seal must be well rounded off and not pointed. Pycnometer opening is wide enough to permit easy filling and emptying, and stopper has fine capillary opening. Walls of bottle meet stopper in rising acute angle of ca 45° so that no depression or groove retaining moisture is formed at this point.

(**j**) *Emptying device for Reischauer pycnometer.*—Bend piece of metal capillary tubing (brass, stainless steel), <2 mm o.d., to ca 45° angle. End to be inserted into pycnometer must reach within 2–3 mm of bottom. Connect other end either to rubber aspirator bulb or to compressed air supply not >5 lbs/sq. in.

(**k**) *Water bath.*—Automatically controlled H_2O bath. If automatic control is not available, use following app. Have H_2O level of bath (5–15 L) reach above neck marks of pycnometer, keep H_2O bath temp. at $20 \pm 0.05°$, and read on accurate thermometer, calibrated to 0.1°. Maintain temp. of H_2O bath by very slow but continuous flow of ice-H_2O from container (2–4 L, contg ice and H_2O). Regulate flow of ice-H_2O by hand. Stir H_2O in bath mechanically and continuously without splashing.

10.069 STANDARDIZATION

(a) *Setting of mill.*—Use malt of following characteristics:

Variety: Malt made from 6-rowed midwestern variety of barley	
Moisture	4.2–4.8%
Ext. in finely ground malt, dry basis	74.0–77.0%
Color of laboratory wort, Lovibond ½″ cell, series 52	not >1.8
Diastatic power, dry basis	not <100
Ratio sol. protein to total protein	36–42
Mealiness: Glassy	not >5%
Mealy	not <90%
Acrospire development: 0–¼ grown	not >5%
¾ to full grown	not <80%
Overgrown	not >5%
Assortment: From malt meeting above specifications, take that portion passing thru 7/64″ screen and remaining on 6/64″ screen for actual stdzg operation.	

Fine grinding.—Weigh 50 g specified malt into mash beaker, grind, and collect in same beaker. Mill must not be in motion when sample is introduced. Transfer to No. 30 std sieve placed on receiving pan and shake in horizontal plane on flat surface 3 min., pausing every 15 sec. long enough to give screen and pan 2 sharp taps on surface over which it is sliding. Transfer and weigh particles remaining on and adhering to screen. Consider mill as having stdzd setting when wt of ground malt remaining on No. 30 sieve is between 4.5 and 5.5 g (9–11%). Stdze mill at least twice yearly. Suitable mechanical shaking device, giving equiv. results, may be used to stdze mill.

Coarse grinding.—Proceed as for *fine grinding.* Consider mill as having stdzd setting when portion of ground malt remaining on No. 30 sieve is between 37 and 38 g (74–76%).

(b) *Reischauer type pycnometer.*—Clean interior and exterior of pycnometer with $Na_2Cr_2O_7$-H_2SO_4 soln, discharge carefully with air, and wash several times with H_2O, then alcohol, and finally ether. To remove last traces of ether vapor and to replace with laboratory air, connect dry metal capillary tubing to vac. and insert into pycnometer 1–2 min. Carefully wipe pycnometer, let stand few min., and det. tare by weighing to 0.2 mg.

Fill with freshly distd H_2O and place in H_2O bath held at $20 \pm 0.05°$. Tap gently to force out air bubbles. After 25 min., remove liquid above mark with capillary pipet provided with small rubber bulb. To make final adjustment of meniscus absorb last quantity of liquid with thin strips of blotting paper; also remove any liquid adhering to inner surface of neck. Adjust H_2O level so that lower part of meniscus rests on mark. Make all adjustments of liquid level within pycnometer neck while holding by neck, without touching body of pycnometer with hands. Keep body of pycnometer submerged during entire period of meniscus adjustment.

Raise pycnometer to room temp. by insertion into H_2O bath kept at exactly that temp., and hold 10 min. Remove pycnometer, carefully dry exterior, and weigh to 0.2 mg. Subtract wt empty pycnometer. Difference between weighings represents H_2O capacity of pycnometer at 20°. Redet. tare wt and H_2O capacity at frequent intervals.

(c) *Boot type pycnometer.*—Clean pycnometer and det. its tare in same way as for Reischauer type. Cool H_2O in ice bath to temp. slightly <20°. Rinse pycnometer once with the cool H_2O, fill, stopper, and dry exterior. Remove stopper, insert thermometer adjusted to 20° in H_2O, and note temp., which should be $20 \pm 0.1°$. If it is not, choose different "filling" temp., which may vary with analyst and season from 19.4° to 19.7° or more. Make subsequent detns, using predetd filling temp. Place cap over stopper and weigh.

Redet. wt, H_2O capacity, and filling temp. at least weekly.

10.070 DETERMINATION

Fine grinding.—Weigh ca 55 g sample (at room temp.) into tared mash beaker and grind thru mill set for stdzd fineness of grind. Collect finely ground malt in same mash beaker, carefully brushing malt particles remaining in mill into mash beaker. Mix, and without delay, place mash beaker with contents on balance accurate to within ±0.05 g under 750 g load and adjust wt malt to 50 ± 0.05 g by removing excess into tared dish for moisture detn.

Coarse grinding.—Weigh 50.5 g sample (at room temp.) into tared mash beaker and grind thru mill set for stdzd coarseness of grind. Collect coarsely ground malt in same mash beaker, carefully brushing particles remaining in mill into mash beaker. Without delay, place mash beaker with contents on balance accurate to within ±0.05 g under 750 g load and adjust wt malt to 50 ± 0.05 g by removing excess.

(a) *Mashing procedure.*—"Mash in" ground malt with 200 ml H_2O at 46° and mix well with glass rod to prevent formation of lumps. Carefully rinse glass rod and wall of beaker with small quantity of H_2O. Note odor of mash and report as aromatic, slightly aromatic, musty, green, stale, etc. Promptly place mash beakers in mashing app. contg H_2O previously heated to 46°, and set stirrers in motion. Place thermometer in each mash beaker. Keep temp. at 45° exactly 30 min. from time beakers were placed in mashing app. Raise mash temp. 1°/min. to 70°. Add 100 ml H_2O, previously heated to 70–71°, and hold mash 60 min. at 70°. (Temp. deviations during mashing procedures should not exceed 0.5°.)

(b) *Conversion.*—Transfer drop of mash with

thin glass rod (ca 3 mm diam.) onto absorbent gypsum plate, **10.068**(**e**), or into one cavity of porcelain plate, and test with drop of $0.01N$ I soln on gypsum plate, or with drop of $0.02N$ I soln, **10.067**(**b**), on porcelain plate. Make tests 5, 7, and 10 min. after 70° is reached, and thereafter if necessary, at 5 min. intervals. Conversion is complete when test drop and I soln produce only yellow stain on gypsum or porcelain plate. Report time of conversion in periods: <5 min., 5–7 min., etc. Time of conversion is not detd on coarsely ground malt.

(**c**) *Cooling and filtration.*—After 60 min., cool mash promptly (within 10–15 min.) to prevailing room temp. Stop stirrers. Remove thermometers after adhering mash particles are rinsed into beaker with H_2O. Remove each beaker with its stirrer from mashing app. Rinse mash particles adhering to stirrer into beaker with H_2O. Dry outside of each beaker, taking care to remove moisture adhering to rim. Without delay, adjust wt of contents of mash beaker to 450.0 ± 0.05 g by adding H_2O.

Stir mash thoroly with glass rod, once when removing beakers from balance pan and again immediately before pouring mash onto filter. (Stirrings must be not <5 min. nor >15 min. apart.) While stirring cooled mash, take care to prevent splashing or spilling. Mix drops adhering to beaker wall into mash by rotary stirring with glass rod.

Pour entire contents of beaker into funnel provided with specified filter paper. Cover funnel with ca 20 cm diam. watch glass during entire filtration. Return first 100 ml filtrate to filter. When no more liquid is present above filter cake, discontinue filtration and remove receiving flask contg wort for later observations and tests. In case of slow running worts, stop filtration after 2 hr. In case of coarse ground malt mash, collect exactly 200 ± 2 ml wort. When filtration is complete, mix wort in receiving flask thoroly by rotary motion. Speed of filtration is normal if filtration is complete (as defined above) within 1 hr after returning the 100 ml filtrate to filter bed; slow, if filtration takes longer. Observe degree of clarity and report as clear, slightly hazy, or hazy.

Remove ca 100 ml wort for detn of color. (Color is not detd on wort from coarsely ground malt.)

(**d**) *Specific gravity.*—Rinse empty pycnometer twice with ca 10 ml wort, and if Reischauer pycnometer is used, remove rinsings each time with emptying device. Fill with wort, place in H_2O bath, and proceed as in **10.069**(**b**). Weigh filled pycnometer within 3 hr of completed filtration. Difference between this wt and that of empty pycnometer represents wort capacity of pycnometer at 20°. Calc. sp. gr. of wort to fifth decimal place, rounding off to 0.00005 or 0.00010, by dividing wt wort by wt H_2O.

No calcn is made of sp. gr. *in vacuo*. If duplicate detns made by same analyst in different beakers differ by more than 2 units in fourth decimal place, repeat entire detn.

(**e**) *Extract.*—Det. ext. yield of wort by reference to sp. gr. values given in **43.003**, and calc. ext. yield of malt by following formulas:

Ext. as-is basis $= P(800 + M)/(100 - P)$, where $P =$ g ext. in 100 g wort (Plato, **43.003**); and $M = \%$ H_2O in the malt.

Ext. dry basis $= (E \times 100)/(100 - M)$, where $E =$ ext. as-is basis; and $M = \%$ H_2O in malt.

Report ext. as-is basis and dry basis to nearest 0.1%.

10.071 Extract in Caramel Malt—Official

Use mill for fine grinding as in **10.068**(**a**). Weigh ca 30.5 g caramel malt, grind, and adjust to 25 ± 0.05 g, removing excess for moisture detn. Weigh ca 25.5 g malt of known moisture, ext., and color, and having diastatic power of 100° L. or over; grind, and adjust to 25 ± 0.05 g. Transfer the 2 portions quantitatively to mash beaker, mash, and det. sp. gr. as in **10.070**(**d**). Det. moisture as in **10.065**(**b**) and **10.066**.

Calc. by following formulas:

$$\text{Total ext.} = P \times (800 + M \text{ in 50 g malt} + M \text{ in 50 g caramel malt})/(100 - P),$$

where $P =$ g ext. in 100 g wort (Plato, **43.003**), and $M =$ moisture (g).

$$\text{Ext. in caramel malt} = (\text{total ext.} - \text{ext. in 50 g malt}) \times 100/50.$$

10.072 Color in Caramel Malt—Official

Use mixed wort obtained for ext. detn, dilg wort enough to make color reading ca 4.0° L. Det. color on dild wort as in **10.074**(a) or (**b**).

Calc. by following formula: Color of caramel malt $= 2[C \times (D+1)] -$ color of malt used for conversion, where $C =$ color reading on dild wort, and $D =$ parts of H_2O to dil. one part of wort.

Report diln used for making color reading. Report color to nearest whole number.

10.073 Color in Black Malt—Official

Use mill for fine grinding as in **10.068**(**a**). As precautionary measure grind small quantity of sample to be analyzed and clean out mill. For detn weigh 5.5 g, grind, and collect all particles by careful brushing of mill.

Weigh 5.00 g on analytical balance, transfer to 600 ml beaker, add 400 ml H_2O at room temp., and heat to boiling in not <15 min. nor >20 min. Boil gently exactly 5 min., cool to room temp., and without delay transfer to 500 ml vol. flask; dil. to vol. with H_2O, mix, and filter thru 32 cm fluted paper. Pipet 10 ml filtrate into 100 ml vol. flask, dil. to vol. with H_2O, and mix.

Det. color of dild wort as in **10.074**(**a**) or (**b**). Calc. color found for this filtrate to same concn of materials used for regular malt mash (12.5 g malt to 100 ml H_2O) by formula:

Color of black malt $= L \times 10 \times 12.5$, where L = color reading on dild filtrate. Report color to nearest whole number.

10.074 Color of Wort—Official

(**a**) *Tintometer method.*—Use Lovibond tintometer, ½″ cell, Series 52, brewers' type, and std daylight lamp (ASTM, D218–34T, 1945, or its spectrophotometric equiv.). Place tintometer in box shield of metal or wood, finished in dull black so as to prevent interference from reflected light. Mount in horizontal position directly in front of artificial daylight lamp. Substitute flashed opal glass for milk glass usually provided with instrument. Have distance between opal glass and daylight lamp such as to project diffused light with absence of glare or shadow upon opal glass and have near surface of daylight filter 6″ from opal glass.

Pour wort into cell within 15 min. after filtration and match against std glasses. (To eliminate errors in matching from additive effects, use no more than 2 slides, preferably 1.) Subdivide down to ⅛° color glasses and report results to nearest tenth. If difficulty is experienced in reading color, filter that portion of wort to be used for color detn separately thru dry paper without filter-aid.

Report color of laboratory wort in ° Lovibond, ½″ cell, Series 52, with a range of 0.2° L.

(**b**) *Dye method.*—*See* **10.004.**

10.075 Protein—Official

Weigh 1.4 g finely ground malt, **10.070,** and proceed as in **2.036.** $\%N \times 6.25 = \%$ protein.

10.076 Wort Nitrogen—Official

Using 25 ml laboratory wort, **10.070**(**c**), det. N as in **10.034.** Calc. as wort N in terms of % malt (dry basis) as follows:

Wort N (% malt, dry basis) = (ml $0.1N$ H_2SO_4 − ml $0.1N$ NaOH) $\times 0.0056 \times \%$ malt ext. (dry basis)/(° Plato of wort $\times$ sp. gr. of wort).

Report to second decimal.

Diastatic Power (*9*)—**Official**

10.077 PREPARATION OF GLASSWARE

Wash all glassware with $Na_2Cr_2O_7$-H_2SO_4 cleaning soln, rinse with tap H_2O at least 4 times, and finally rinse with H_2O at least twice. Thoroly dry digestion flasks.

10.078 REAGENTS

(**a**) *Acetate buffer soln.*—Dissolve 68 g NaOAc $.3H_2O$ in 500 ml $1N$ HOAc and dil. to 1 L with H_2O.

(**b**) *Fehling soln.*—Stdze as in **29.036–29.037.** Check soln from time to time by detg its oxidizing value against std soln of invert sugar, **29.035**(**c**), as in **29.036.**

(**c**) *Alkaline ferricyanide soln.*—$0.05N$. Dissolve 16.5 g dry $K_3Fe(CN)_6$ and 22 g anhyd. Na_2CO_3 in H_2O, and dil. to 1 L with H_2O. Soln keeps its strength for long period if stored in dark glass bottle in dark.

(**d**) *Sodium thiosulfate soln.*—$0.05N$. Dissolve 12.41 g clear crystals of $Na_2S_2O_3.5H_2O$ and 3.8 g $Na_2B_4O_7.10H_2O$ (as preservative) in 100–200 ml H_2O and dil. to 1 L. If redistd CO_2-free H_2O (second distn being made after addn of small quantity of alk. $KMnO_4$ soln to first distillate, to destroy all traces of org. matter) is used in dilg soln, it will retain its normality long time, whereas with ordinary H_2O it is likely to deteriorate slowly on standing. Check the $Na_2S_2O_3$ against the $K_3Fe(CN)_6$ soln as follows: To 10 ml of the $K_3Fe(CN)_6$ soln add 25 ml of the HOAc reagent, 1 ml of the 50% KI soln, and 2 ml of the sol. starch indicator. Titr. with the $Na_2S_2O_3$. (Exactly 10 ml $Na_2S_2O_3$ soln should be required to completely discharge blue starch-I color.) Stdze $Na_2S_2O_3$ soln against pure I if necessary.

(**e**) *Acetic acid reagent.*—200 ml HOAc, 70 g KCl, and 20 g $ZnSO_4.7H_2O$/L.

(**f**) *Potassium iodide soln.*—50%. Dissolve 50 g KI in H_2O, add 1 drop NaOH soln (1+1), and dil. to 100 ml with H_2O. (Adding the NaOH soln prevents or substantially delays deterioration of the soln, with liberation of I, on standing; soln must be colorless.)

(**g**) *Starch soln.*—Have final concn represent 2 g sol. starch (weighed on dry basis) in 100 ml soln. Use sol. starch, according to Lintner, special for diastatic power detn, with solubility at least 1:50 in hot H_2O, that contains no dextrins, contains <0.75% reducing substances calcd as maltose, and has moisture content of 10–12%. Freshly made 2% soln must have pH of 4.5–5.5 without adjustment with buffer. Subsequent batches of starch, when tested on a malt of ca 100° Lintner (dry basis) having other characteristics as specified under detn of ext. in malt, must show variation no $> \pm 3°$ Lintner from value obtained by using original starch in parallel detn. Test addnl batches of starch, when purchased, in parallel with starch in use. Permit no variation $> \pm 3°$ Lintner. In no case may cumulative correction as referred to original starch, approved above, amount to >5° Lintner. (Starch meeting these specifications is available from Merck & Co., Rahway, N. J.)

Macerate starch with just enough cold freshly distd H_2O to form smooth, thin paste (not >5% of final vol.). Pour, with constant stirring, into boiling freshly distd H_2O representing not <ca 75% of final vol. of starch soln, at such rate that

boiling does not cease. Continue boiling 2 min. after thin paste is completely added. Quickly add to beaker addnl 10% of final vol. of cold, freshly distd H_2O and transfer mixt. quantitatively to g-s. vol. flask; mix by inverting flask, wash down neck of flask, and cool to 20° before adding buffer soln. Add 2 ml buffer soln for each 100 ml of final vol. of starch soln and dil. to mark. Mix again by inverting flask and keep tightly stoppered at 20° until used.

(**h**) *Soluble starch indicator.*—1% sol. starch in 30% NaCl soln. Prep. sol. starch suspension and pour slowly into boiling H_2O. Add NaCl and dil. to vol. (Soln should be transparent and colorless.)

10.079 DETERMINATION

Grind separately not >25.5 g malt as in **10.070.** Collect finely ground malt in mash beaker, carefully brushing in malt particles remaining in mill. Without delay, adjust wt contents to 25 ±0.05 g. Transfer quantitatively to container (ca 1 L) in which infusion is to be made. Add 500 ml 0.5% NaCl soln at 20° and close container. Let infusion stand 2.5 hr at 20 ±0.2° and agitate by rotating at 20 min. intervals. Take care that in agitation of malt suspension as small quantity of grist as possible is left adhering to inner surface of flask above level of the H_2O. (Do *not* invert flask to mix; gentle whirling of contents without splashing on sides of container is sufficient.) Filter infusion by transferring entire charge to 30–32 cm fluted filter (S&S No. 588 or equiv.) in 185 mm funnel. Return first 50 ml filtrate to filter. Collect filtrate for 3 hr after H_2O and ground malt were first mixed. Prevent evapn during filtration as far as possible by placing watch glass over funnel and some suitable cover around stem of funnel, resting on neck of receiver.

Immediately dil. 20 ml of this infusion to 100 ml with 0.5% NaCl soln at 20°, transfer 10 ml dild infusion to 250 ml vol. flask, and bring to 20°. Add 200 ml buffered starch soln from fast-flowing pipet, all at 20°. Mix soln by rotating flask during addn. Keep "starch infusion" mixt. at 20 ±0.1° exactly 30 min., timed on stop-watch from time addn of starch was begun. Add 20 ml 0.5*N* NaOH rapidly and mix well by whirling flask. Dil. to mark at 20° and mix thoroly.

Det. reducing power by (**a**) Fehling soln modification, or (**b**) ferricyanide modification:

(**a**) *Fehling soln modification.*—Boil 10 ml of the Fehling soln and 10 ml H_2O in 200 ml erlenmeyer. (For heating soln, elec. plate is preferable to gas flame.) Add, from buret, ca ⅔ of quantity of above digested starch soln probably required and boil 15–20 sec., rotating constantly. Remove from heat. If still decidedly blue, add more soln, boil ca 10 sec., and again observe color. When blue color is almost discharged, and after boiling gently ca 2 min., add 3 drops *1% aq. methylene blue soln.* Continue boiling and add more soln until 0.1 ml, or even 1 drop, upon boiling, discharges blue color. (Color becomes violet-lavender as end point nears.)

Repeat titrn, adding at once almost whole quantity of digested starch required, and proceed to end point as directed. Designate quantity of digested starch soln required to reach end point in this second titrn as A. Interrupt boiling as little as possible after indicator is added, so that flask remains filled with steam, preventing much access of air. (Upon cooling, blue color usually returns.)

Prep. blank by processing exactly as in **10.079,** par. 2, except add the 0.5*N* NaOH to malt infusion before adding the starch soln. To 10 ml of the Fehling soln and 10 ml H_2O add a vol. of this blank soln equal to final vol. of digested starch soln required in above detn. Boil and again det. end point as in the detn. Designate quantity of digested starch soln used as B.

(**b**) *Ferricyanide modification.*—Pipet 5 ml dild digested starch soln into 125 ml erlenmeyer. Pipet exactly 10 ml of the $K_3Fe(CN)_6$ soln into the soln, and immerse flask in vigorously boiling H_2O bath. Have surface of liquid in flask 3–4 cm below surface of boiling H_2O. Let flask remain in boiling H_2O bath *exactly* 20 min.; then cool under running H_2O, and add 25 ml of the HOAc soln, **10.078(e),** with thoro mixing. Add 1 ml of the KI soln, followed by 2 ml of the starch indicator, and mix thoroly. Titr. with the 0.05*N* $Na_2S_2O_3$ to complete disappearance of blue color (10 ml buret is recommended). Designate ml 0.05*N* $Na_2S_2O_3$ used as A.

Prep. blank by proceeding exactly as in **10.079,** par. 2, except to add the 0.5*N* NaOH to malt infusion before adding starch soln. Det. reducing power of blank as in preceding par. Designate ml 0.05*N* $Na_2S_2O_3$ used for blank as B.

10.080 CALCULATION OF DIASTATIC POWER

(**a**) *Fehling soln modification.*—Degrees Lintner (°L) as-is basis $= (5000/A) \times (B/A)$;

°L dry basis = (°L as-is basis $\times 100)/(100 - M)$, where A and B have same meaning as in **10.079(a),** and M = % moisture.

In above formula, $5000/A$ is apparent diastatic power, which must be modified by fraction representing ratio of blank titrn to original titrn, which measures influence of starch in detn.

Report °L as-is and dry basis to nearest whole number. °L × 4 = maltose equiv. (M.E.) (g reducing substances, calcd as maltose, produced by 100 g malt in half-hour digestion of sol. starch at 20° under conditions specified in method).

M.E. as-is basis $= (20{,}000/A) \times (B/A)$; M.E. dry basis = (M.E. as-is basis $\times 100)/(100 - M)$, where A, B, and M are as defined above.

(b) *Ferricyanide modification.*—Degrees Lintner (°L) as-is $=(B-A)\times 23$, where A and B have same meaning as in **10.079(b)**. Calc. °L dry-basis from this as in (a) and report to nearest whole number.

Maltose equiv. (M.E.) as-is basis $=(B-A)\times 92$. Calc. M.E. dry basis from this as in (a). M.E. $/4=$°L.

When conditions given in method are followed, net quantity of ferricyanide, after correcting for blank, $\times 23=$diastatic power in Degrees Lintner as-is basis. °L$\times 4=$maltose equiv. Therefore, ferricyanide equiv.$\times 92=$maltose equiv.

Alpha-Amylase (*10*)—Official

10.081 REAGENTS

(a) *Special starch.*—Use Merck's soluble Lintner starch, special for diastatic power detn. *See* **10.078(g)**.

(b) *Beta-amylase.*—Use special β-amylase powder free from α-amylase made by the Wallerstein Laboratories, Wallerstein Square, Mariners Harbor, Staten Island 3, N.Y. This prepn has been stdzd to 2000°L and should comply with following specifications: At addn level used, there must not be variation $>5\%$ in dextrinization of std malt infusion when 1 and 3 day old substrates are compared. Further, substrate prepd by adding twice the level of β-amylase indicated must not deviate by $>5\%$ from that prepd with recommended level after 24 hr standing. Store powder in tightly closed bottle in refrigerator. To avoid moisture condensation on cold enzyme prepn, let bottle warm to room temp. before opening.

(c) *Stock iodine soln.*—Dissolve 5.50 g I crystals (ACS) and 11.0 g KI in H_2O, and dil. to 250 ml with H_2O. Store in dark bottle and make fresh soln monthly.

(d) *Dilute iodine soln.*—Dissolve 20.0 g KI in H_2O, add 2.00 ml of the stock I soln, and dil. to 500 ml with H_2O. Series of 13×100 mm test tubes contg 5 ml of the dil. I soln must be made up beforehand and adjusted to 20° in readiness for testing. All-glass automatic pipet such as the Machlett type is recommended for rapidly dispensing this soln.

(e) *Buffer soln.*—Dissolve 120 ml HOAc and 164 g anhyd. NaOAc in H_2O, and dil. to 1 L.

(f) *Sodium chloride soln.*—0.5%. Dissolve 5 g reagent NaCl in 1 L H_2O. This soln need not be made up in vol. flask.

(g) *Buffered limit-dextrin (alpha-amylodextrin) substrate.*—Prep. suspension of 10.00 g (dry wt) Merck's soluble starch in cold H_2O and pour slowly into boiling H_2O. Boil with stirring 1–2 min., cool, and add 25 ml of the buffer soln and 250 mg of the β-amylase dissolved in small amount of H_2O. Dil to 500 ml with H_2O, sat. with toluene, and store at ca 20° for not <18 hr nor >72 hr before use.

10.082 APPARATUS

(a) *Constant temperature bath.*—Set at 20 ± 0.05°.

(b) *Reference color standard.*—Use the special Alpha-Amylase Color Disk (Catalog No. 620-S5) made by Hellige Inc., 877 Stewart Ave., Garden City, Long Island, N. Y.

(c) *Comparator.*—Use either std Hellige comparator (Catalog No. 607) or pocket comparator (Catalog No. 605) with prism attachment (Catalog No. 605-A). Illuminate comparator with 100 watt frosted lamp mounted in such manner that direct rays from the lamp do not shine in operator's eyes. Place lamp 6″ from rear opal glass of comparator. Slight differences in color discrimination between different operators are minimized by use of prism attachment, maintenance of 6–10″ reading distance between eye and comparator, and by experience gained with continued practice.

(d) *Comparison tubes.*—Use precision bore square tubes with 13 mm viewing depth. Place tube filled with distd H_2O behind color disk.

The α-amylase color disk is correct only when used with specified 13 mm viewing depth. Precision bore square tubes are specified to obviate need for individual calibration of test tubes and to insure use of std viewing depth. The 13 mm precision square tubes are supplied as std equipment with Hellige Comparator and are also used with Coleman Universal spectrophotometer. They may be secured from either Hellige Inc., distributors of Coleman instrument, or Fischer and Porter Co., Hatboro, Pa.

10.083 DETERMINATION

(a) *Preparation of malt infusion.*—Ext. 25 ± 0.05 g finely ground malt exactly as in **10.079**, par. 1, using 500 ml 0.5% NaCl soln. Dil. 20 ml malt infusion to 100 ml with 0.5% NaCl soln at 20°.

(b) *Dextrinization.*—Transfer 20.0 ml substrate soln at 20° to 50 ml erlenmeyer, add 5 ml 0.5% NaCl soln, and again adjust to 20°. Add 5 ml dild malt infusion at 20°, blowing it in and counting time from instant first of the dild malt infusion reaches starch substrate in flask. After 10 min. reaction time, add 1 ml of the hydrolyzing mixt. to 5 ml of the dil. I soln at 20°, shake, pour into the 13 mm square tube, and compare with α-amylase color disk in comparator. At appropriate intervals remove addnl 1 ml aliquots of the hydrolyzing mixt., add to dil. I soln, mix, and compare with color disk until α-amylase color is reached. Take care to keep tubes contg reaction

mixt. plus I from changing temp. while comparing colors. If color comparisons are made immediately following addn of reaction mixt. to I, there will be essentially no temp. change and no change in color.

During initial stages of reaction the 1 ml sample need not be measured precisely before addn to dil. I soln. As end point approaches, make addn accurately with 1 ml pipet. (Use fast flowing pipet such as 1 ml bacteriological pipet for withdrawing 1 ml aliquot.) Blow contents of pipet into I soln. Near end point, take readings every 0.5 min. on the min. or half min. In case two readings 0.5 min. apart show that one is darker than α-amylase color disk and other is lighter, then end point is recorded at nearest 15 sec. Shake out 13 mm square tube used for color comparison between successive readings.

For accuracy and convenience it is desirable that dextrinization times fall between 10 and 30 min. With malts of low α-amylase activity it may be necessary to use 10 ml of the dild infusion. In this case, do not add 5 ml of the NaCl soln. Final vol. of reaction mixt. should always be 30 ml.

10.084 CALCULATION OF ALPHA-AMYLASE ACTIVITY

From time interval in min. necessary for dextrinization, T, and wt malt in g represented by infusion aliquot taken, W, calc. α-amylase units. An α-amylase unit is defined as quantity of α-amylase which will dextrinize soluble starch in presence of excess of β-amylase at rate of 1 g/hr at 20°.

$$20°\ D.U.\ \text{(as-is basis)} = 24/(W \times T);$$
$$20°\ D.U.\ \text{(dry basis)} = D.U.\ \text{(as-is)} \times 100/(100 - M);$$

where $M = \%$ moisture in sample and $24 =$ wt starch employed (0.4 g) multiplied by 1 hr (60 min.).

Example: $W = 0.05$ g; $T = 20$ min.; 20° $D.U.$ (as-is) $= 24/(0.05 \times 20) = 24$.

Report dextrinizing units to nearest 0.1 unit.

CEREAL ADJUNCTS

10.085 **Sampling—Official**—*See* **10.054(a), (b), and (c)**

10.086 **Preparation of Sample—Official**—*See* **10.055**

10.087 **Physical Characteristics—Procedure**

(**a**) *Accidental foreign particles.*—Before proceeding with laboratory detns remove any accidental foreign particles from sample. Report presence and quantity.

(**b**) *Color.*—Spread evenly suitable portion of sample and observe against white background Report as white, cream, yellow, buff, gray, brown.

(**c**) *Odor.*—Det. after shaking sample in closed container. Report as clean and normal, moldy, musty, rancid, or other foreign odor.

(**d**) *Husks, germs, and foreign seeds.*—Classify and report in %.

(**e**) *Mold.*—Det. by visual inspection and report as none, trace, considerable.

(**f**) *Weevils, larvae, etc.*—Det. presence or absence by visual inspection. Report as none, very few, few, considerable; indicate if alive or dead.

Moisture—Official

Air Oven Method (103–104°)

10.088 APPARATUS—*See* **10.064**

10.089 DETERMINATION

Grind as in **10.070** and proceed as in **10.066.**

Oil or Petroleum Ether Extract—Official

10.090 REAGENT

Petroleum ether.—Skellysolve F. Initial boiling temp., 35–38°; dry-flask end point, 52–60°; at least 95% distg under 54°, and not >60% distg under 40°; sp. gr. at 60°F., 0.630–0.660; appearance, colorless; evapn residue, not >0.0011 g/100 ml; doctor test, sweet; copper-strip corrosion test, noncorrosive; only trace of unsatd compounds permitted; residue in distg flask, neutral to Me orange; blotter strip odor test, odorless within 12 min.; aromatic compounds, no nitrobenzene odor; saponification value, <1.0 mg KOH/100 ml.

Make distn test according to ASTM method D216-54 and make blank detn by evapg 250 ml with ca 0.25 g stearin or other hard fat (previously brought to constant wt by heating) and drying as in actual detn. Blank must be not >3 mg.

10.091 DETERMINATION

Weigh accurately 5–10 g sample ground as in **10.070.** Without previous drying, ext. in Soxhlet or other suitable extractor with the petr. ether for not <6 hr. Filter ext. thru small, hardened paper into weighed vessel, washing paper finally with small portion of hot fresh solvent. Distill or evap. solvent at temp. not >100° and dry vessel contg residual extractive matter in air oven 1 hr at 100–105°. Report as % oil to second decimal.

Extract—Official

10.092 APPARATUS

Same as in **10.068** except that mill may be of any suitable type.

10.093 STANDARDIZATION

Setting of mill.—Use sample of rice or grits with moisture content not $>12\%$. Grind enough sample to obtain at least 51 g ground portion. Det. fineness of grinding as in **10.069(a)**. Fine grinding of rice should show 40 ± 2.5 g ($=80 \pm 5\%$) and grits 35 ± 2.5 g ($=70 \pm 5\%$) of ground portion passing thru std sieve.

10.094 DETERMINATION

Grind enough sample so that at least 21 g is obtained. Grind ca 31 g malt made mainly from 6-rowed barley of Manchurian type, conversion time not >7 min., diastatic power 100–120° Lintner. Det. ext. of malt simultaneously with that of the cereal.

Mash in 20 ± 0.05 g sample (with exception of flaked corn and flaked rice) and 5 ± 0.05 g of the ground malt with 200 ml H_2O at 46°. Mix well with glass rod, place on wire gauze over flame, and bring to boil in not <10 min. nor >15 min., stirring constantly. Boil grits and rice gently 30 min., and refined grits 10 min., avoiding burning, spattering, and excessive frothing. During boiling, stir mash and keep vol. constant by adding boiling H_2O every 15 min. After boiling, cool to 46° and add 25 ± 0.05 g of the remaining ground malt. When ext. is detd on flaked corn or flaked rice, do not boil, but mash in 20 g unground sample and 30 g ground malt with 200 ml H_2O at 46°. Mix well to prevent formation of lumps, rinse inner walls of beaker, promptly place mash beakers in mashing app. contg H_2O previously heated to 46°, and set stirrers in motion. Hold at 45° 30 min. from time mash beakers were placed in app. Raise mash temp. 1°/min. until 70° is reached. Add 100 ml H_2O, previously heated to 70–71°, and hold mash 60 min. at 70°. (All temps refer to mash, not H_2O bath temp. Temp. deviation during mashing should not be $>0.5°$.)

To test conversion, transfer drop mash to one cavity of porcelain plate and add drop $0.02N$ I soln, **10.067(b)**; conversion is complete when test drop and I soln give yellow color. Report time of conversion in periods: <15 min., 15–30, 30–45, 45–60, incomplete at 60 min. Cool, filter as in **10.070(c)**, and det. sp. gr. and corresponding ext. as in **10.070(d)** and **(e)**.

10.095 CALCULATION

$$\text{Total ext.} = P \times (800 + M \text{ in 60 g malt} + M \text{ in 40 g sample})/(100 - P)$$

where P = ext. from Plato's table, **43.003**, and $M = \%$ moisture.

$$\text{Ext. in sample} = (\text{total ext.} - \text{ext. in 60 g malt}) \times 100/40.$$

10.096 Crude Fat or Ether Extract—Official—*See* **22.033**

10.097 Protein—Official—*See* **2.036**

Multiply results by 6.25.

10.098 Ash—Official—*See* **13.006**

10.099 Crude Fiber—Official—*See* **22.040**

HOPS

10.100 Sampling—Official

(Oregon sampler, Bates divider, and excellent sampling procedure are described in "Hop Inspection Manual Covering the Determination of Leaves and Stems and Seeds in Hops," Instruction No. 918(GR)-1, July 1, 1951, available from Grain Division, Agricultural Marketing Service, Washington 25, D.C.)

(**a**) *Unpressed hops.*—Draw equal portions from 5 or 10 different parts of heap, from surface as well as from different depths, until ca 200 g is obtained. Place sample in suitable container such as tin can having screw- or friction-type cover, moisture-proof plastic bags, or jars.

(**b**) *Baled hops.*—Use Oregon sampling device or sharp knife to cut 200 g samples from at least 10% of bales in shipment under 100 bales and square root of the no. of bales if >100, avoiding sampling at press seam. Place each bale sample in sep. container. (If chemical tests are to be made, store in tin cans having friction or screw tops, not cartons, paper bags, wrapping paper, or wooden boxes.) Take sufficient equal quantity from each bale sample so that total of 100 g is combined in one composite sample. (Use of Bates divider gives most accurate results.)

10.101 Physical Examination (*11*)—First Action

(**a**) *Leaves and stems.*—Pick out, with tweezers or forceps, stems and leaves from 20 ± 0.01 g sample. Det. wt leaves and stems to 1 decimal place. Use remainder of sample for seed detn, if required.

(**b**) *Size and condition of cones.*—Report size according to following classification:

	LENGTH (INCHES)
Large	$2\frac{1}{4}$–3
Medium	$1\frac{1}{4}$–2
Small	$\frac{3}{4}$–1

Report condition of cones as unbroken, partly broken, much broken.

(**c**) *Lupulin.*—Break 10 cones into longitudinal halves and examine lupulin grains thereby exposed in good light, preferably daylight, as to quantity, color, and condition. Report quantity as plentiful, fairly plentiful, scarce. Report color

as lemon-yellow, orange-yellow, brownish. Report condition as sticky, fairly sticky, not sticky.

(**d**) *Seeds.*—Dry portion remaining from leaf and stem detn or sep. 20 g sample, in oven 3–6 hr at >100° or 2 hr at 114–116° (long enough to eliminate stickiness). (If rapid drying is necessary place sample in 2 foot square muslin cloth, immerse in bowl of MeOH or trichloroethylene 1 min., press out excess liquid by hand, using rubber gloves for protection, and spread cloth contg hops on screen to dry in air or over steam radiator.)

Fold portions of the dried hops in dry square of muslin and rub between hands to crush petals completely; then empty finely pulverized material onto 4×20 wire mesh screen to sep. petal substance. Continue this process until portion left on screen consists mainly of seeds and rachillae, sep. these by rolling seeds off large sheet of sandpaper into tared dish, weigh, and report % by wt to 1 decimal place.

(**e**) *Color and luster.*—Det. color and luster on whole cones and refer findings to predominating character of sample. Report color as greenish-yellow, yellowish-green, pale green, olive-green, dark green. Describe presence of quantities of differently colored cones as: small, medium, or large quantity of ______ cones present, using appropriate color terminology, such as brownish, reddish, etc.

(**f**) *Aroma.*—Rub several cones between hands. Report odor as aromatic, mildly aromatic, abnormal. Use term flowery to describe exceptionally fine aroma. Use proper designations, such as musty, cheesy, etc., to describe abnormal odors.

10.102 Preparation of Sample for Chemical Analysis—Official

Grind hop samples immediately before analysis. Let samples stored in refrigerator come to room temp. before grinding.

Grind 50–75 g sample in No. 2 Universal food chopper, using 12-tooth cutter, or No. 3 Russwin food chopper (available from Russell and Erwin Division, American Hardware Corp., 10 Franklin Square, New Britain, Conn.). Discard first 5 or 10 g. Place polyethylene bag over discharge of chopper so that hops pass directly into bag. Pass hops evenly and slowly thru grinder, taking care to avoid choking orifices so as to prevent undue heating of hops. Thoroly mix ground portion into homogeneous mass and store in air-tight container in cool, dark place. (In some cases definite quantities of ground portions from several samples may be mixed together, and analyses run, in duplicate, on mixed portion.) For accurate results in analysis for resins, detn must be completed on same day as grinding, since resins are subject to oxidation.

10.103 Moisture—Official

Use one of following procedures, which are listed in order of accuracy:

(1) Me cyclohexane or *n*-heptane distn method with 10.00 g sample.

(2) Vac. drying 3 hr at 60° at 22–23″ Hg.

(3) Drying 1 hr at 103–104°.

For (2) and (3) use 2.5 g ground sample in 55 mm weighing bottle or Al dish, or 5 g ground sample in 70 mm dish. (Quantity of hops and dimensions of dish used are important for accurate results.) Report results in % to first decimal place, and state method used.

Resins (*11*)—Official

10.104 REAGENT

Lead acetate soln.—Dissolve 1 g $Pb(OAc)_2.3H_2O$ in absolute MeOH, add 0.1 ml HOAc, and dil. to 100 ml with absolute MeOH.

DETERMINATION

10.105 *Soft Resins*

Accurately weigh 10 g well-mixed ground hops, **10.102,** into 500 ml erlenmeyer or other suitable flask; pipet in 200 ml absolute MeOH cooled to 20°; stopper tightly with rubber stopper or cork covered with tinfoil; and shake vigorously by machine or hand 10 min. Filter ca 100 ml ext. into 100 ml vol. flask, cool to 20°, and add enough filtrate to bring to mark. Pipet 50 ml of this ext. (equiv. to 2.5 g hops) into 250 ml Squibb separator contg 200 ml 2% NaCl soln. (Instead of filtration, centrifuge may be used. Cool to 20° and pipet 50 ml clear, supernatant ext. into separator.)

Ext. mixt. by shaking with 4 successive 45–50 ml portions of petr. ether, **10.090.** Release any pressure developed in separator thru cock. Drain lower aq. layer into 200 ml erlenmeyer after each shaking, and drain petr. ether layer into plain glass funnel fitted with soft, rapid, 11 cm paper (S&S No. 589), collecting filtrates in 250 ml vol. flask. Return aq. soln in erlenmeyer to separator, carefully wash out flask with few ml petr. ether, and transfer this washing to separator. Repeat extn 3 times. Wash paper and funnel thoroly with petr. ether until all traces of resins dissolve, and dil. filtrates to 250 ml at 20°. Stopper, and mix thoroly.

Return extd. aq. MeOH mixt. to separator for later detn of hard resins. Transfer 100 ml petr. ether ext. (equiv. to 1 g hops) with pipet or buret into tared 150 ml side-arm distn flask or Soxhlet extn flask. Evap. solvent by distn on H_2O bath at 60° until 5–10 ml remain. Disconnect flask and insert stopper fitted with glass delivery tube reaching almost to bottom of flask but not touch-

ing liquid. Flow dry CO_2 thru tube and apply vac. to side arm. Keep flask immersed to neck in boiling H_2O until constant wt is obtained. Displace CO_2 by air and weigh to nearest mg after standing near balance 30 min.

10.106 *Alpha Acids*

(**a**) *Preliminary titration.*—Transfer 40 ml of the petr. ether ext. remaining from soft resin detn to small beaker and evap. solvent on H_2O bath. Transfer residue to ca 25 ml centrifuge tube with small portions absolute MeOH to vol. of 8 ml, warm to 60°, and add 1.5–2 ml of the $Pb(OAc)_2$ soln. Centrifuge liquid and transfer drop of clear supernatant onto piece of filter paper close beside drop of *10% $(NH_4)_2S$ or Na_2S soln* so that margins of drops interpenetrate. Continue adding the $Pb(OAc)_2$ soln (0.5 ml at time) and centrifuging until 1 drop supernatant produces definite brown color with sulfide when spotted on filter paper. Multiply number of ml $Pb(OAc)_2$ soln used by 2.5 and use this quantity for pptn of alpha acids in main test.

(**b**) *Precipitation of alpha acids.*—Pipet 100 ml of the petr. ether ext. (equiv. to 1 g hops) remaining from soft resin detn into 150 ml side-arm distn flask or other suitable flask, and remove solvent by distn on H_2O bath at 60°, using vac. and driving off last 5–10 ml with suction as in **10.105** but at not >60°. (Vac. oven may also be used for drying.) Dissolve residue in flask by warming with small quantity absolute MeOH and wash into 50 ml beaker until 18–20 ml is obtained. Cover beaker with watch glass and immerse in H_2O bath at 60° ca 5 min. Add calcd quantity of the $Pb(OAc)_2$ soln (as detd by the preliminary titrn) slowly and with constant stirring, and digest mixt. 5 min. at 60°. Let beaker stand at room temp. until ppt settles, and test clear supernatant for excess Pb. If result is negative, heat beaker and contents again to 60° and add 0.5 ml of the $Pb(OAc)_2$ soln. Repeat operations until slight but obvious excess of Pb is indicated by definite brown color in sulfide test. Let soln stand at room temp. 30 min. and collect ppt in tared gooch. Remove all traces of ppt from beaker to crucible with rubber policeman and wash thoroly with MeOH. Dry crucible and contents to constant wt in oven at 100–101° (1 hr). % alpha acids = wt Pb salt $\times 0.631 \times 100$.

If rapid estimation of alpha acid content is desired, omit preliminary titrn, and begin with 100 ml petr. ether ext. Proceed as above and ppt alpha acids directly by adding 7 ml of the $Pb(OAc)_2$ soln. (This quantity is usually enough to ppt alpha acids from hops having av. composition of 16–19% soft resins.)

In detg alpha acid content of hops with exceptionally high or exceptionally low content of soft resins, 1–2 ml more or less of the $Pb(OAc)_2$ reagent will be required for satisfactory results. Care should be taken to avoid use of excess $Pb(OAc)_2$ as this tends to redissolve pptd alpha acids, but insufficient $Pb(OAc)_2$ may cause greater errors than when excess is used, because insufficient $Pb(OAc)_2$ leaves large quantities of alpha acids in soln.

10.107 *Beta Fraction*

% beta fraction = % soft resins − % alpha acids.

10.108 *Hard or Gamma Resins*

Shake aq. MeOH mixt. remaining from **10.105** with four 40 ml portions absolute ether. Use first portion to wash filter paper (previously used to filter petr. ether soln of soft resins) and to rinse flask used in sepn of aq. portion from previous petr. ether extn. Collect combined ether exts in suitable flask and remove ether by distn. Dry flask and contents on H_2O or steam bath. Dissolve residue in 5 ml absolute MeOH and again heat until dry on H_2O or steam bath with constant and rapid twirling of flask to spread ext. uniformly on interior of flask. Dry in oven 1 hr at 105° and weigh when cool.

Dissolve hard resins in absolute ether, followed by small quantity absolute MeOH, and discard mixt., being careful not to lose any NaCl crystals. Dry flask, which contains slight trace of NaCl, with suction and again weigh. Difference in wt before and after treatment with ether represents hard resins. % hard resins = difference in wt $\times 100/2.5$.

10.109 *Total Resins*

% total resins = % hard resins + % soft resins.

BREWING SUGARS AND SIRUPS

10.110 Color and Clarity—First Action

(**a**) *Clarity.*—Observe degree of clarity of unfiltered "10% soln," **10.111**(**a**). Report as clear, slightly hazy, or hazy.

(**b**) *Color.*—Free "10% soln," **10.111**(**a**), from suspended matter and, if possible, from haze by filtration thru dry paper. Det. color as in **10.004** or **10.074**. Report as color of "10% soln" to nearest 0.1° Lovibond.

10.111 Extract—Official

(**a**) *Preparation of "10% soln."*—Accurately weigh ca 50 g well-mixed representative sample, dissolve in warm H_2O, transfer quantitatively to 500 ml vol. flask, and dil. to vol. at 20°. Mix thoroly.

(**b**) *Determination.*—With suitable pycnometer det. sp. gr. of soln at 20/20°, as in **10.069**(**b**) or

(c). Obtain corresponding ext. from **43.003.** Calc. % ext. in original sample, E, from following formula:

$$E = P \times B \times 500/W,$$

where P = ext. of dild sample; B = sp. gr. of dild sample; and W = actual wt (ca 50 g) of sample taken. Report to first decimal place.

(c) *Degrees Baumé.*—Obtain degrees Baumé (Modulus 145) equiv. to ext. of original sample (b) from **43.003.**

10.112 Non-Extract (Apparent Water)—Official

Obtain by subtracting ext. of original sample, **10.111(b)**, from 100.

10.113 Fermentable Extract (*12*)—Official

(a) *Regular fermentation method.*—Ferment 250 ml of the "10% soln" of the sample, **10.111(a)**, with equiv. of 5 g washed, active brewers' compressed yeast 48 hr at 15–25° or until fermentation is complete. In case of refined sugars and sirups, such as corn sirup, add to soln, before fermenting, 0.8 g K_2HPO_4 crystals, 1 g $NH_4H_2PO_4$, and 0.5 g dried yeast ext., stdzd for bacteriological culture media purposes, as nutrients. If such nutrient material needs to be added, redet. ext. of the "10% soln" after adding nutrient material, but before adding yeast. Use fermentation flasks equipped with either H_2O, Hg, or acid seals, and shake flasks several times a day during fermentation. When fermentation is complete, filter soln thru dry paper, refiltering first 20–30 ml filtrate. (Filtrate should be clear, but not necessarily brilliant.) Det. real ext. in filtrate as in **10.021(a)**, after removal of alcohol.

Use following formulas for calcn:

Fermentable ext. (ext. basis) $= (p - n) \times 100/P$;

and

Fermentable ext. (as-is basis) $= (p - n) \times E/P$;

where P = ext. of "10% soln" before addn of any nutrients; p = ext. of "10% soln" before fermentation; n = real ext. of "10% soln" after fermentation; and E = ext. of original sample, **10.111(b)**.

If nutrients have not been used (as in case of malt sirups), $p = P$, and will have been detd in **10.111(b)**, and no redetn before fermentation is required. Report to first decimal place.

(b) *Rapid fermentation method.*—Proceed as in (a), but instead of 250 ml of the "10% soln" of sample, **10.111(a)**, use equiv. of 32 g fresh compressed brewers' yeast or liquid yeast that has been de-watered by suction on büchner (more precise results are obtained by washing yeast with the "10% soln" of the sample before final suction filtration). For refined sugars and sirups, use nutrients as in (a). Ferment mixt. at room temp. (20–23°) and stir continuously with 4 blade glass stirrer (ca 2" diam.) at 100–120 rpm until fermentation is complete (4–5 hr). As evapn can be an important variable, keep stirring and time at minimum. Filter, det. real ext., and calc. as in (a).

Yeast autolysis can affect results by contributing solids to fermented liquid. Effect may be checked by detg pH of fermented liquid, its alcohol content, and calcd original gravity, **10.022.** If pH is high and original gravity is appreciably higher than ext. of "10% soln" originally detd, yeast autolysis has probably occurred and detn should be repeated with fresh yeast.

10.114 Protein—Official

(a) Transfer 25 ml of the "10% soln," **10.111(a)**, to Kjeldahl digestion flask, and proceed as in **10.034.**

% protein (N × 6.25) in original as-is sample

$$= 100 \times (\text{ml } 0.1N\ H_2SO_4 - \text{ml } 0.1N\ \text{NaOH}) \times 0.0014 \times 6.25 \times 500/(25 \times W)$$

where W = actual wt sample used in prepg the "10% soln." Report to second decimal place.

(b) Det. N as in **29.019,** and calc. protein, using factor N × 6.25.

10.115 Diastatic Power—Official

(Malt sirups only)

Transfer 10 ml of the "10% soln," **10.111(a)**, to 100 ml vol. flask and dil. to vol. at 20° with H_2O. Transfer 10 ml of the "1% soln" so prepd to 250 ml vol. flask, bring to 20°, add 200 ml buffered starch soln at 20°, **10.078(g)**, and proceed as in **10.079,** last 4 sentences in second par., beginning with words: "Mix soln" and ending "mix thoroly." Follow Fehling soln modification, **10.079(a)**.

Calc. on as-is basis according to formula:

Degrees Lintner (as-is basis)

$$= (5000 \times B \times 50)/(A \times A \times W)$$

where A = ml digested starch soln required to reach end point in detn; B = ml digested starch soln required to reach end point in blank; and W = wt sirup used to prep. "10% soln."

10.116 Iodine Reaction for Unconverted Starch—Official

Use the "10% soln," **10.111(a)**, and proceed as in **10.038.**

10.117 Acidity—Official

Transfer 100 ml of the "10% soln," **10.111(a)**, to suitable beaker or flask and proceed as in **10.025,** second par., or **10.026,** beginning "titr.

potentiometrically." Calc. and report results as follows (W = actual wt sample, in g, used to prep. "10% soln"):

(a) In terms of ml $1N$ NaOH/100 g original sample, as-is basis:

$$\text{Acidity} = \text{ml } 0.1N \text{ NaOH consumed} \times 500/(10 \times W).$$

Report to first decimal.

(b) In terms of "lactic acid" as % original sample as-is basis:

$$\text{Acidity} = \text{ml } 0.1N \text{ NaOH consumed} \times 0.009 \times 500/W.$$

Report to second decimal.

10.118 Hydrogen-Ion Concentration (pH)—Official

Using the "10% soln," **10.111(a)**, proceed as in **10.027**.

10.119 Ash—Official

Proceed as in **29.012** or **29.013**.

Total Reducing Sugars (*13*)—Official

10.120 *Munson-Walker General Method*

Transfer 50 ml of the "10% soln," **10.111(a)**, to 250 ml vol. flask. Clarify, if necessary, with alumina cream or neutral $Pb(OAc)_2$ soln only (never basic $Pb(OAc)_2$), and dil. to vol. at 20° with H_2O. Mix thoroly and either centrifuge or filter until clear. If $Pb(OAc)_2$ soln was used for clarification, remove excess Pb with dry $Na_2C_2O_4$. Filter, and det. reducing sugars on 10 ml aliquot as in **29.039–29.040, 29.054,** or **29.065.** Calc. results in terms of invert sugar for invert sirups and sugars; dextrose for corn sugars and sirups; and maltose for malt sirups. If character of sample is in doubt, express reducing sugars as dextrose.

% Reducing sugar, as is $= 25M/W$, where M is mg sugar from appropriate column of **43.011,** and W is g sample used to prep. "10% soln".

10.121 *Lane-Eynon General Volumetric Method*

Dil. 50 ml of the soln, clarified as in **10.120,** to 100 ml and proceed as in **29.036–29.037, 29.053,** or **29.064,** referring titer to **43.017** or **43.018.** Calc. results as in **10.120.**

10.122 Dextrose—Official

To 5 ml aliquot of soln prepd as in **10.120,** add 15 ml H_2O and proceed as in **29.178** or **29.182.**

10.123 Other Determinations— *See* Chap. **29**

WORT—OFFICIAL

10.124 Preparation of Sample

Store 1 gallon wort 12–15 hr at 4–7°; then filter all but last portion of this sample at 4–7° thru paper of types specified in **10.068(f)**. If filtrate is not brilliant after first filtration, return to filter, but do not use filter-aid. (Some worts cannot be filtered brilliantly clear.) To prevent spoiling, keep sample in refrigerator, and if necessary, place in beer bottles and pasteurize. Mix sample well to insure uniformity before removing portion for analysis.

10.125 Specific Gravity

Proceed as in **10.018** or **10.070(d)**. Report to fifth decimal place.

10.126 Original Extract or Original Gravity

From **43.003,** find ext. corresponding to sp. gr. detd at 20/20°. Report as ° Plato (g/100 g) to second decimal place.

10.127 Fermentable Extract

(a) *Regular method.*—Ferment 250 ml of the wort with equiv. of 5 g washed, active brewers' compressed yeast 48 hr at 15–25°, or until fermentation is complete. Use either H_2O, Hg, or acid seal to prevent evapn. Filter soln and det. real ext. in filtrate as in **10.021(a)** after removal of alcohol.

Calc. % by wt of fermentable ext. as in **10.113.** Report to second decimal place.

Calc. also real degree of fermentation as in **10.023.** Report to first decimal place.

(b) *Rapid method.*—(Dets fermentability of worts contg up to ca 14% ext. in 4–5 hr within 0.3 – 0.1% of attenuation limit.)

To 200 ml wort in 400–600 ml glass beaker add 32 g fresh, compressed, washed brewers' lager yeast or liquid yeast that has been dewatered by suction on büchner (more precise results are obtained by washing yeast with wort to be pitched before final suction filtration). Keep mixt. at room temp. (20–23°) and stir continuously with glass stirrer until fermentation is complete (4–5 hr). Filter mixt. thru ordinary filter paper, refiltering first 20–30 ml filtrate. (Filtrate should be clear, but not necessarily brilliant. As evapn can be important variable, keep stirring and time at minimum. Four-blade glass stirrer (ca 2″ diam.) operating at 100–120 rpm is satisfactory. Abnormal effects due to autolysis are likely to be indicated by too high pH of final beer and high calcd original gravity compared to actual original gravity. *See* **10.113(b)**.) Det. real ext. and calc. as in (**a**).

10.128 Iodine Reaction—*See* **10.038**

10.129 Total Acidity—*See* **10.025** and **10.026**

10.130 Hydrogen-Ion Concentration (pH)—*See* **10.027**

10.131 Color

Prep. sample as in **10.124.** If prepd wort has developed haze or sediment that would interfere with detn, clarify by centrifuging or filtering without use of filter-aid. Indicate such clarification in report. Det. depth of color of prepd sample as in **10.074.** Report results in degrees Lovibond, ½″ cell, Series 52, to first decimal place.

10.132 Protein

Prep. sample as in **10.124.** If prepd sample shows sediment, mix thoroly to insure perfect distribution of sediment before removing portion for analysis. To det. protein in the brilliant wort (free from any haze or sediment), reclarify prepd sample by centrifuging or filtering without use of filter-aid. Indicate such clarification in report.

Pipet 25 ml prepd sample, measured at 20°, into 800 ml Kjeldahl flask and proceed as in **10.034.**

10.133 Total Reducing Sugars

Prep. sample as in **10.124.** If prepd wort contains appreciable quantities of suspended matter, remove by centrifuging or filtering. Transfer 50 ml sample to 250 ml vol. flask, dil. to vol. at 20°, and mix thoroly. In general, worts do not require clarification for detn of reducing sugars, but if clarification is necessary, proceed as in **10.120.** Det. reducing sugars in 10 ml of this soln by Munson-Walker method as in **29.065.** Or dil. 50 ml of this soln with H_2O to 100 ml and use Lane-Eynon method as in **29.064.** Express results as % maltose.

YEAST

Liquid and Pressed Yeast

Sampling (*14*)—Procedure

10.134 APPARATUS

Dry, wide-mouth 1 L containers with suitable cover or dry Mason jars with covers are acceptable for collecting samples. Jar should hold ca twice vol. of original sample.

10.135 COLLECTING PRIMARY SAMPLE

(**a**) *Bottom fermenting yeast.*—(1) From small fermenters from which the yeast is collected in cans or tubs, collect at least five 100 ml portions of the yeast slurry at intervals as it is forced out thru bung hole into brink. (2) From large tanks from which the yeast is removed by pump, collect 5–10 100 ml portions of the yeast slurry from sampling cock at regular intervals on discharge side of the yeast pump.

(**b**) *Top fermenting yeast.*—Push aside upper fluffy layer of yeast and take portion from under layer. Collect at least five 100 ml portions from various parts of tank. (For routine work top fermenting yeast is usually sampled after it has been skimmed into yeast buggy. Mix thoroly before sampling.)

(**c**) *Any liquid yeast from small tanks or tubs.*—Mix contents of tub thoroly to uniform consistency, taking care to blend in heavier deposits on bottom of vessel and to remove gases. Take at least 500 ml sample from this mixt.

(**d**) *Pressed yeast.*—Remove portions from different parts of the cake—from surface as well as from center—and collect ca 150 g in 1 L beaker. Weigh to nearest 0.1 g. Prep. slurry by adding H_2O at rate of ca 3 parts H_2O to 1 part pressed yeast. Again weigh to nearest 0.1 g. With stirring rod, break up yeast portions and stir until liquid suspension is completely uniform.

10.136 PRESERVATION OF SAMPLES

To prevent changes in analytical results due to autolysis and fermentation, proceed with examination immediately after samples have been obtained. Keep sample at 2° or below.

10.137 PREPARATION OF LABORATORY SAMPLE

Mix primary composite sample thoroly and transfer working quantity to sep. container. If lumps or particles of trub are present, pass thru sieve entire bulk sample of liquid yeast, which should amount to not <500 ml if yeast requires screening, or slurry prepd from ca 150 g pressed yeast. Make sure all lumps and particles are broken up and forced thru sieve. Recover, by scraping, any liquid or solids adhering to sieve, and reincorporate them with sieved sample. Mix well by stirring.

Total Solids—Official

Alcohol Method

10.138 APPARATUS

(**a**) *Sieve.*—Approx. 100-mesh.

(**b**) *Moisture oven.*—*See* **10.064**(**b**).

(**c**) *Weighing dish.*—Glass or Al, at least 65 mm i.d., with cover, and glass stirring rod of such length that it fits within covered dish.

10.139 REAGENTS

(**a**) *Alcohol.*—Pure alcohol or MeOH, or alcohol denatured with completely volatile liquid.

such as denatured alcohol Nos. 1, 1-A, 2-B, 3-A, 12-A, 13-A, 23-A.

(b) *Sand.*—Use clean, sharp sand. Wash with H_2O and dry overnight at 105°. Cool in desiccator. Keep in closed container. Loss of wt of 5 g of this sand when dried 3 hr at 105° must not be >0.005 g.

10.140 DETERMINATION

Place ca 5 g dry sand in weighing dish. Weigh dish together with sand, cover, and stirring rod. Transfer to weighing dish ca 10 g well-mixed liquid yeast or yeast slurry from pressed yeast, cover, and weigh to nearest 0.001 g. Remove cover and add 5 ml alcohol. Mix thoroly with stirring rod. Drop rod into weighing dish. Dry 3 hr (±2 min.) at 105°. Cover, cool in desiccator, and weigh.

For liquid yeast calc. drying loss of aliquot used as % and report as total solids to first decimal place. For pressed yeast calc. according to following formula: % total solids $= D \times S \times 100/W \times P$, where P = wt (g) pressed yeast used for prepg slurry; S = total wt (g) yeast slurry; W = wt (g) slurry aliquot before drying; and D = wt (g) slurry aliquot after drying.

Dried Yeast

Total Solids—First Action

Vacuum Oven Method (15)

10.141 APPARATUS

(a) *Metal dish.*—Diam. ca 55 mm, height ca 15 mm, provided with inverted slip-in cover fitting tightly on inside. Or, diam. ca 65 mm, height ca 20 mm, provided with slip-over cover fitting tightly on outside.

(b) *Air-tight desiccator.*—$CaCl_2$, reignited CaO, or Drierite are satisfactory drying agents.

(c) *Vacuum oven.*—Connected with pump or vac. system capable of maintaining pressure equiv. to 50 mm or less of Hg, and provided with thermometer passing into oven with bulb near samples. Connect H_2SO_4 gas-drying bottle to oven to admit dry air when releasing vac.

10.142 DETERMINATION

Weigh accurately ca 2 g well-mixed sample in covered dish, previously dried at 98–100°, cooled in desiccator, and weighed soon after attaining room temp. Loosen cover (do not remove) and heat 5 hr at 98–100° at pressure of 50 mm Hg or less. Admit dry air into oven to bring to atmospheric pressure. Immediately tighten cover on dish, transfer to desiccator, and weigh soon after attaining room temp.

Air Oven Method (16)

10.143 DETERMINATION

Weigh accurately ca 2 g well-mixed sample in covered dish, **10.141(a)**, previously dried at 100 ±1°, cooled in desiccator, and weighed soon after attaining room temp. Loosen cover (do not remove) and heat 16 hr at 100±1°. Tighten cover, transfer to desiccator, and weigh soon after attaining room temp.

BREWERS' GRAINS* (17)—OFFICIAL

10.144 SAMPLING

(a) *Wet brewers' grains.*—Collect by means of scoop numerous small samples at uniform intervals during emptying of tub, so that at end of operation composite sample of 25–30 lbs is obtained in clean, dry bucket. From mash filters collect numerous small samples in similar manner at equal time intervals from grain conveyor. Mix thoroly and quarter grains carefully so as to obtain representative sample of 3–4 lbs. Place reduced sample in suitable container with screw or friction type cover, add few drops of toluene as preservative, close tightly, and refrigerate.

(b) *Dry brewers' grains.*—*See* **10.054.** Take great care in sampling dry brewers' grains for analysis, particularly for feed, as it is very difficult to obtain truly representative sample. Because brewers' grains are composed of large husks and small, heavy particles, there is usually difference in composition at different levels of container.

If brewers' grains are in sacks, sample not <2% of the sacks, using trier as long as height of sacks. Quarter sample down to ca 0.5 lb for laboratory sample.

If brewers' grains are in car, unsacked, it is practically impossible to obtain representative sample because of segregation at bottom of fine heavy material, which is higher in protein than lighter material at top.

Store sample in refrigerator pending laboratory analysis. On each sample container show date of sampling; name of company owning grains at time of sampling; and brew, lot, car, or reference number or letter for identification. Before making analysis, mix grains thoroly.

10.145 **Preliminary Drying (Wet Brewers' Grains)**

Weigh accurately (±0.1 g) ca 1000 g quartered wet brewers' grains on weighed, shallow, galvanized Fe or Al tray so that layer is not >¼" thick. After spreading, moisten grains with little toluene to inhibit fermentation during drying.

* For examination of brewers' grains for feeding purposes see Chap. 22.

Dry in oven at 55–60°, or overnight in air by means of fan and heater, until grains appear air-dry. Note accurately (± 0.1 g) wt dried grains and store in moisture-proof container. Thoroly mix dried sample and grind finely 100 g, as in **10.070**. Keep ground portion in moisture-proof container.

10.146 Moisture

(**a**) *On sample after preliminary drying (when available and soluble extracts are determined).*—Use 5–10 g accurately weighed and ground sample and proceed as in **10.066**. Calc. % moisture in dried grains (W). Calc. % moisture, M, in original wet grains by following formula: $M = [(W \times D) + 100(G - D)]/G$, where $G =$ wt wet grains before preliminary drying, $D =$ wt grains after preliminary drying, and $W = \%$ moisture in grains after preliminary drying.

(**b**) *On sample in wet condition (when only soluble extract is determined).*—Use ca 15 g sample, accurately weighed into 70 mm Al dish, and dry first at temp. $<60°$ until air-dry; then dry 3 addnl hr as in **10.066**.

(**c**) *On dry brewers' grains.*—Proceed as in (**a**) and calc. % moisture.

Available Extract

10.147 *Wet Brewers' Grains*

(**a**) *Apparatus.*—Mash beakers and counter wts, mashing app., filter paper, funnels, flasks, pycnometers, emptying device, and H_2O bath. *See* **10.068**.

(**b**) *Preparation of sample.*—*See* **10.145**. *Preparation of finely ground malt.*—*See* **10.070**.

(**c**) *Mashing procedure.*—*See* **10.094**. Proceed as for flaked corn and flaked rice.

(**d**) *Cooling and filtration.*—*See* **10.070(c)**.

(**e**) *Specific gravity.*—*See* **10.070(d)**. Det. corresponding Plato values from **43.003**.

(**f**) *Calculation.*—Use following formulas:

$$\text{Total ext.} = P \times (800 + W \text{ in } 60 \text{ g malt} + W \text{ in } 40 \text{ g dried grains})/(100 - P),$$

where $P =$ g ext. in 100 g wort (Plato), and $W =$ moisture (g).

$$\% \text{ available ext. in wet grains, dry basis} = \frac{(E \text{ in mixt.} - E \text{ in } 60 \text{ g malt}) \times 10{,}000}{40(100 - M \text{ of dried grains})}$$

where $E =$ ext., and $M = \%$ moisture.

% available ext. in wet grains, as-is basis $=$ (available E, dry basis) $(100 - M$ of wet grains$)/100$, where $E =$ ext., and $M = \%$ moisture.

10.148 *Dry Brewers' Grains*

Preparation of sample.—Grind finely 100 g sample and proceed as in **10.147**. Calc. ext. as in **10.095**.

Soluble Extract (Wet Brewers' Grains)

10.149 *On Sample After Preliminary Drying*

(**a**) *Mashing procedure.*—"Mash in" in mash beaker 25 ± 0.05 g unground sample with 350 ml H_2O at 70°. Place mash beakers in mashing app., **10.068(c)**, (**d**), contg H_2O previously heated to 70–71°, and set stirrers in motion. Hold mash 60 min. at mash temp. of $70 \pm 0.5°$.

(**b**) *Cooling and filtering.*—*See* **10.070(c)**. Make mash to 425 g.

(**c**) *Specific gravity.*—*See* **10.070(d)**. Det. corresponding Plato values from **43.003**.

(**d**) *Calculation.*—Use following formulas:

$$\% \text{ sol. ext. in wet grains, dry basis} = P(M \text{ of dried grains} + 1600) \times 100/(100 - P)(100 - M \text{ of dried grains}$$

$$\% \text{ sol. ext. in wet grains, as-is basis} = (E, \text{ dry basis})(100 - M \text{ of wet grains})/100$$

where $P =$ g ext. in 100 g wort (Plato, **43.003**), $E =$ ext., and $M = \%$ moisture.

10.150 *On Sample in Wet Condition*

Mashing method.—Using 100 ± 0.05 g well-mixed and quartered brewers' grains and 300 ml H_2O at 71°, proceed as in **10.149**. Make mash to 450 g.

Calculation.—Use following formulas:

$$\% \text{ sol. ext., as-is basis} = P(M + 350)/(100 - P);$$

$$\% \text{ sol. ext., dry basis} = E \times 100/(100 - M);$$

where $P =$ g ext. in 100 g wort (Plato, **43.003**), $M = \%$ moisture content, and $E =$ sol. ext., as-is.

Report sol. ext. on as-is and dry basis in %, to first decimal.

10.151 Soluble Extract (Dry Brewers' Grains)

Proceed as in **10.149**, but use following formula:

$$\% \text{ sol. ext., as-is basis} = P(1600 + M)/(100 - P);$$

$$\% \text{ sol. ext., dry basis} = E \times 100/(100 - M);$$

where $P =$ g ext. in 100 g wort (Plato, **43.003**), $E =$ sol. ext., as is, and $M = \%$ H_2O in dry brewers' grains.

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(*3*) J. Lab. Clin. Med. **11**, 981(1926); Am. Soc. Brewing Chemists Proc. **1951**, 27; **1954**, 160; **1955**, 214; **1956**, 240; **1957**, 165.
(*4*) J. Assoc. Offic. Agr. Chemists **28**, 448 (1945).
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(*7*) Am. Soc. Brewing Chemists Proc. **1950,** 166.

(*8*) J. Assoc. Offic. Agr. Chemists **27,** 372 (1944).

(*9*) Am. Soc. Brewing Chemists Proc. **1941,** 90; **1942,** 124.

(*10*) Ibid. **1951,** 153.

(*11*) Ibid. **1941,** 130.

(*12*) J. Assoc. Offic. Agr. Chemists **28,** 441 (1945).

(*13*) Ibid. **28,** 435(1945).

(*14*) Am. Soc. Brewing Chemists Proc. **1946,** 130; **1948,** 137; ASBC Methods of Analysis, 6th Ed., 1958, p. 199.

(*15*) J. Assoc. Offic. Agr. Chemists **39,** 738 (1956).

(*16*) Ibid. **40,** 446(1957).

(*17*) Am. Soc. Brewing Chemists Proc. **1942;** **1941,** 114–6.

11. Beverages: Wines *(1)*

11.001 Physical Examination—Procedure

Note and record following: (*a*) Whether container is "bottle full"; (*b*) appearance, whether bright or turbid and presence of sediment; (*c*) condition when opened, whether still, gaseous, or carbonated; (*d*) color and depth of color; (*e*) odor, whether vinous, foreign, or acetous; and (*f*) taste, whether dry, sweet, vinous, foreign, or acetous.

11.002 Preparation of Sample—Official

Remove any gas by pouring sample back and forth in beakers. Filter wine, regardless of appearance. Det. immediately sp. gr. and those ingredients that are subject to change, such as alcohol, sugars, and acids.

11.003 Specific Gravity—Official

Det. sp. gr. at 20/20° by pycnometer as in **9.011,** or by small, accurately graduated hydrometer.

Alcohol

11.004 *By Volume from Specific Gravity—Official*

Measure 100 ml sample into 300–500 ml distn flask, noting temp., and add 50 ml H_2O. Attach flask to vertical condenser by means of bent tube, distill almost 100 ml, and dil. to vol. of 100 ml at same temp. (Foaming, which sometimes occurs, especially with young wines, may be prevented by adding small quantity of antifoam material.) For wines that contain abnormal quantity of HOAc neutralize exactly with 1*N* NaOH soln (calcd from acidity detn, **11.029**) before proceeding with distn (unnecessary for wines of normal taste and odor). Proceed as in **9.011,** at room temp. if desired, and obtain corresponding % alcohol by vol. from **43.021.**

11.005 *By Volume from Refraction (Rapid Method)—Official*

Det. immersion refractometer reading of distillate obtained in **11.004** and find corresponding % alcohol from **43.022.**

By Volume Using Etienne Tube (2)—Procedure

11.006 APPARATUS

Etienne tube.—See Fig. 21. Clean frequently and dry. (Available from Scientific Glass Apparatus Co., Bloomfield, N.J.)

11.007 REAGENTS

(**a**) *Solvent.*—Mix 70 ml Pentasol (synthetic amyl alcohol), 28 ml toluene, and 1.8 ml dil. HCl, **9.018(a)**. Shake well until acid completely dissolves.

(**b**) *Carbon.*—Activated carbon (Darco S-51).

11.008 PREPARATION OF SAMPLE

Place 30–40 ml sample into convenient size flask and add enough C (ca 0.5 g) to fairly well decolorize sample. Stopper, and shake ca 30 sec. Pour entire contents onto dry filter paper. Refilter until clear.

11.009 DETERMINATION

Pipet 10 ml sample, directly or prepd as in **11.008,** into tube. Accurately adjust bottom of meniscus to coincide with 10 ml mark. Remove any excess sample on sides of tube above mark with swab or roll of filter paper. Add 20 ml reagent to 30 ml mark. Stopper tube tightly with rubber stopper and invert tube number of times with moderate shaking to mix intimately (ca 2

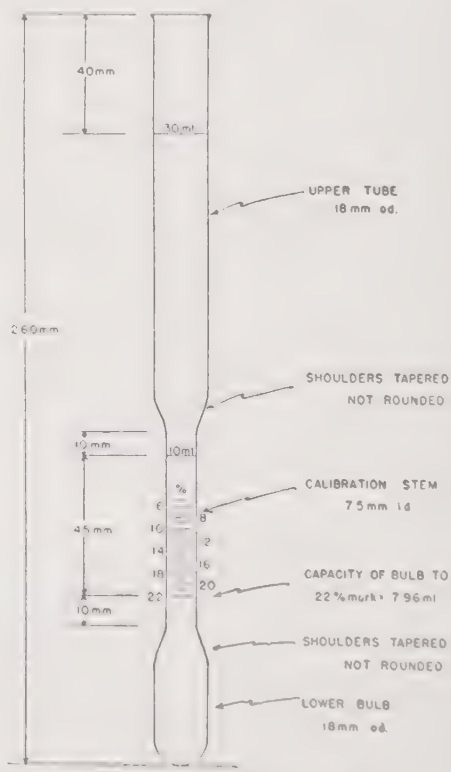

FIG. 21.—ETIENNE TUBE

min.). Stand tube upright and let sep. When sepn is complete, shake down globules of lower soln that adhere to sides by rotating tube. When settling and drainage are complete (usually ca 5 min.), read % alcohol where meniscus between the 2 layers falls on calibration mark. Repeat mixing and settling and read again.

11.010 TEMPERATURE CORRECTION

Correct for effect of temp. as follows: Add to or subtract from observed % alcohol 0.07% alcohol for each degree > or <78° F. When temp. is >78°F subtract; when temp. is <78°F, add.

11.011 *By Weight—Official*

From **43.023**, obtain % alcohol by wt in distillate corresponding to % alcohol by vol., multiply by sp. gr. of distillate, and divide by sp. gr. of sample.

11.012 Glycerol in Dry Wines—Official

(At no time during evapns should area of dish exposed to bath be greater in circumference than that covered by liquid in dish, arranged by floating dish in bath.)

(**a**) *By direct weighing.*—Evap. 100 ml sample to ca 10 ml in porcelain dish on H_2O bath held at 85–90°. Treat residue with ca 5 g fine sand and 4–5 ml *milk of lime* (contg 15 g CaO/100 ml) for each g ext. present and evap. almost to dryness. Treat moist residue with 50 ml alcohol, 90% by vol., remove substance adhering to sides of dish with spatula, and rub whole mass to paste. Heat mixt. on H_2O bath to incipient boiling with constant stirring, and decant liquid thru filter into small flask. Wash residue repeatedly by decantation with 10 ml portions hot 90% alcohol until filtrate totals ca 150 ml. Evap. filtrate to sirupy consistency in porcelain dish, transfer residue to small, g-s., graduated cylinder with 20 ml absolute alcohol, and add three 10 ml portions anhyd. ether, shaking thoroly after each addn. Let stand until clear, pour thru filter, and wash cylinder and filter with mixt. of 2 parts absolute alcohol to 3 parts anhyd. ether, also pouring wash liquor thru filter. Evap. filtrate to sirupy consistency, dry 1 hr at 98–100°, weigh, ignite, and reweigh. Loss on ignition = wt glycerol.

(**b**) *By oxidation with dichromate.*—Evap. 100 ml sample to ca 10 ml in porcelain dish on H_2O bath held at 85–90°. Treat residue with ca 5 g fine sand and 4–5 ml *milk of lime* (contg 15 g CaO/100 ml). Proceed as in **28.075**, beginning "evap. almost to dryness, with frequent stirring . . . " except to dil. soln of glycerol after treatment with Ag_2CO_3 and $Pb(OAc)_2$ to vol. of 100 ml instead of 50 ml. Observe precautions given concerning temp. at which all evapns are to be made.

11.013 Glycerol in Sweet Wines—Official

If ext. is >5 g/100 ml, heat 100 ml to boiling in flask and treat with successive small portions of *milk of lime* until wine becomes first darker, then lighter in color. Cool, add 200 ml alcohol, let ppt settle, filter, and wash with alcohol. Treat combined filtrate and washings as in **11.012.**

11.014 Glycerol-Alcohol Ratio—Official

Express as X:100. X = % glycerol by wt × 100 / % alcohol by wt.

11.015 **Extract—Official**

(**a**) *By specific gravity of dealcoholized wine.*—Calc. sp. gr. of dealcoholized wine, D, by following formula: $D = S + 1 - A$; S = sp. gr. of sample, **11.003**; and A = sp. gr. of alc. distillate, **11.004.**

From **43.003** ascertain % by wt of ext. in dealcoholized wine corresponding to value of D. This figure × value of D = g ext./100 ml wine.

(**b**) *By evaporation.*—(1) *In dry wines, extract content less than 3 g/100 ml.*—In 75 ml flat-bottom Pt dish, ca 85 mm diam., evap. 50 ml sample on H_2O bath to sirupy consistency. Heat residue 2–5 hr in drying oven at 100°, cool in desiccator, and weigh as soon as room temp. is reached.

(2) *In sweet wines.*—If ext. content is 3–6 g/100 ml, treat 25 ml sample as in (1). If ext. is >6 g/100 ml, accept result obtained as in (a), and attempt no gravimetric detn because of inaccurate results obtained by drying levulose at high temp.

11.016 Non-Sugar Solids (Sugar-Free Extract)—Official

Subtract quantity of reducing sugars before inversion, **11.017,** plus sucrose, if present, from ext., **11.015.**

11.017 **Reducing Sugars—Official**

(**a**) *Dry wines.*—Place 200 ml sample in porcelain dish, exactly neutralize with 1N NaOH, calcg quantity required from detn of acidity, **11.029,** and evap. to ca 50 ml. Transfer to 200 ml vol. flask, add enough neutral $Pb(OAc)_2$ soln, **29.021**(**d**), to clarify, dil. to mark with H_2O, shake, and filter thru folded paper. Remove Pb with dry K oxalate and det. reducing sugars as in **29.039.**

(**b**) *Sweet wines.*—Approximate sugar content by subtracting 2 from ext., **11.015,** and use such quantity of sample that aliquot taken for Cu reduction contains not >240 mg invert sugar. Proceed as in (**a**).

11.018 **Sucrose—Official**

(**a**) *By reducing sugars before and after inversion.*—Proceed as in **9.063**(**b**).

(**b**) *By polarization.*—Polarize before and after

inversion in 200 mm tube, as in **29.025** or **29.026**, portion of filtrate obtained in **11.017**. In calcg % sucrose do not fail to take into consideration relation of wt sample contained in 100 ml to normal wt for instrument.

11.019 Commercial Glucose—Procedure

Polarize portion of filtrate obtained in **11.017**, after inversion in 200 mm jacketed tube at 87°, as in **29.034**. In calcg % glucose do not fail to take into consideration relation of wt sample contained in 100 ml to normal wt for instrument.

11.020 Ash—Official

Proceed as in **29.012** or **29.013**, using residue from 50 ml sample. Char carefully (decrepitation), and ash at not $>550°$.

11.021 Alkalinity of Ash—First Action

Evap. 10 ml sample to dryness in Pt dish and ash at 550°. If solid content of sample is high, it may be necessary to moisten ext. with ether and to burn off carefully over flame to prevent spattering. If any C remains, add few ml H_2O, dry, and again heat to 550°. To ash add 10 ml $0.1N$ H_2SO_4, bring acid in contact with all of the ash, and fill dish ca $\frac{3}{4}$ full of boiling H_2O. Cool, add 4 drops *Me purple* (available from Fisher Scientific Co.) or Me orange, and immediately titr. excess acid with $0.1N$ NaOH. Express alky as ml $0.1N$ H_2SO_4 required to neutralize ash from 100 ml sample.

11.022 Alkalinity of Water-Soluble Ash—Official

Ext. ash obtained in **11.020** with successive small portions of hot H_2O until filtrate totals ca 60 ml and proceed as in **29.016**. Express alky as ml $0.1N$ acid required to neutralize H_2O-sol. ash from 100 ml sample.

Phosphorus

11.023 *Gravimetric or Volumetric Method—Official*

Dissolve ash, **11.020**, in 50 ml boiling HNO_3 $(1+9)$, filter, wash paper, and proceed with combined filtrate and washings as in **2.019** or **2.022**. If residue ignites without difficulty in **11.020**, no free H_3PO_4 need be suspected.

Spectrophotometric Method (3)—Official

11.024 REAGENTS

(a) *Molybdate reagent.*—Dissolve 25 g NH_4 molybdate in 500 ml H_2O, add 140 ml H_2SO_4, and dil. to 1 L with H_2O.

(b) *Sodium bisulfite soln.*—Dissolve 150 g $NaHSO_3$ in H_2O and dil. to 1 L with H_2O. Keep well stoppered and filter if cloudy.

(c) *Sodium sulfite soln.*—Dissolve 200 g Na_2SO_3 in H_2O and dil. to 1 L with H_2O. Keep well stoppered and filter if cloudy.

(d) *Sulfonic acid reagent.*—Dissolve 1.25 g pulverized 1-amino-2-naphthol-4-sulfonic acid in 490 ml of the $NaHSO_3$ soln and shake. Add Na_2SO_3 soln in 5 ml portions until soln is clear (ca 25 ml). Check against std phosphate solns weekly.

(e) *Phosphate std soln.*—Dissolve 0.4393 g pure anhyd. KH_2PO_4 in H_2O and dil. to 1 L with H_2O. 1 ml = 0.1 mg P or 0.229 mg P_2O_5.

11.025 DETERMINATION

Pipet 10 ml sample into Pt dish and evap. to dryness in 100° oven. Carefully char over low flame to avoid spattering, and ignite in muffle at 525°. Dissolve ash in 10 ml $0.1N$ H_2SO_4, transfer to 100 ml vol. flask, and dil. to mark. Pipet 25 ml soln into 100 ml vol. flask, add 50 ml H_2O, 10 ml molybdate reagent, and 4 ml sulfonic acid reagent, shaking after each addn, and dil. to mark. Det. transmittance at 830 mμ (red sensitive phototube) exactly 10 min. after adding the sulfonic acid reagent.

Det. P from std curve prepd from 0, 1.0, 2.0, 3.0, 4.0, and 5.0 ml std phosphate soln. Proceed with detn as above. Temp. of sample should be $\pm 3°$ from temp. at which calibration curve was detd.

11.026 Sulfates—Official—*See* 28.078

11.027 Chlorides—Official

To 100 ml dry wine or 50 ml sweet wine add enough Na_2CO_3 to make distinctly alk. Evap. to dryness, ignite at dull redness, cool, ext. residue with hot H_2O, acidify H_2O ext. with HNO_3 $(1+4)$, and det. Cl as in **6.066** or **6.068**.

11.028 pH—Official

Let pH meter with glass and calomel electrodes warm up before use according to instructions of manufacturer. Check meter with freshly prepd, satd, aq. soln of K bitartrate, **42.007(b)**. Adjust meter to read 3.55 at 20°, 3.56 at 25°, or 3.55 at 30°.

Rinse electrodes free of bitartrate by dipping in H_2O and then in sample. Place electrodes in fresh sample, det. temp., and read pH to nearest 0.01 unit.

11.029 Acidity—Official

Add ca 1 ml phthln to ca 250 ml recently boiled H_2O in large porcelain dish. Neutralize with $0.1N$ NaOH. Heat portion of wine to be titrd to incipient boiling to remove CO_2 and transfer 5 ml for deeply colored red wine or 20 ml for white wine to dish. Titr. rapidly to distinct pink.

Express results in terms of following acids:

Grape wine, 1 ml 0.1*N* NaOH = 0.0075 g tartaric acid; apple wine, 1 ml 0.1*N* NaOH = 0.0067 g malic acid; berry and other wines, 1 ml 0.1*N* NaOH = 0.0070 g citric acid.

11.030 Total Volatile Acidity—Official

(**a**) Heat 50 ml sample rapidly to incipient boiling in 500 ml distn flask connected with condenser and pass steam thru until 15 ml distillate requires only 2 drops 0.1*N* NaOH for neutralization. Boil H_2O used to generate the steam several min. to expel CO_2 before connecting steam generator with distn flask. Titr. rapidly with 0.1*N* NaOH, using phthln. (Color should remain ca 10 sec.) Express results as HOAc: 1 ml 0.1*N* NaOH = 0.0060 g HOAc.

(**b**) (*4*) Introduce 10 ml sample, freed from excess CO_2 by pouring back and forth between large beakers, into inner tube of modified Hortvet distn app., Fig. 22. (Preferably use sufficiently large inner Sellier tube (ca $1\frac{1}{2} \times 8''$) and large distn trap.) Place 150 ml recently boiled hot H_2O in outer flask. Connect with slanting or vertical straight tube condenser and distill, by heating outer flask, into 300 ml erlenmeyer, marked at ca 80 ml, until 80 ml distillate collects. If wine is new or is charged with CO_2, bring distillate to boiling, boil 30 sec., and titr. hot with 0.1*N* NaOH, using phthln.

As alternative, adjust H_2O flow thru condenser so that condensate is received hot. Distill at such rate as to obtain the 80 ml in ca 10 min. For wines with abnormally high HOAc content, continue distn and titr. each succeeding 10 ml distillate until not >1 drop 0.1*N* alkali is required to reach neutral point. If wine is free of CO_2, or has been previously freed from CO_2 by heating to incipient boiling and cooling, or by shaking thoroly *in vacuo* in flask connected to H_2O aspirator, distillate may be titrd cold. Use 10 ml buret graduated in 0.05 or 0.02 ml.

11.031 Volatile Acidity—Exclusive of SO_2 (*5*)—First Action

Pipet 50 ml sample into 100 ml vol. flask. If white, add 2–3 drops phthln, and neutralize to decided pink with clear satd $Ba(OH)_2$ soln; if red, add enough $Ba(OH)_2$ soln to bring mixt. to ca pH 8, using phthln as external indicator. Let mixt. stand 30 min. and keep at phthln end point by adding more $Ba(OH)_2$ if necessary. Dil. to 100 ml, mix, and filter rapidly thru fluted, rapid paper (such as Whatman No. 2). Pipet 20 ml filtrate into inner Sellier tube of Hortvet type app., using larger tube, and add 1 ml H_2SO_4 (1+3); place 150 ml recently boiled hot H_2O in outer flask, and distill 100 ml. Titr. with 0.1*N* NaOH, using phthln.

11.032 Fixed Acidity—Official

Calc. fixed acidity by multiplying total volatile acidity by 1.25 for tartaric, 1.12 for malic, or 1.17 for citric acid (hydrate), and subtracting product from total acidity.

11.033 Total Tartaric Acid (*6*)—Official

Neutralize 100 ml sample with 1*N* NaOH, calcg from acidity, **11.029**, number of ml 1*N* alkali necessary. If >10 ml alkali is added, evap. to ca 100 ml. Add, to neutralized soln, 0.075 g tartaric acid for each ml 1*N* alkali added. It is essential that the tartaric acid be pure; recrystallize if necessary. After tartaric acid dissolves, add 2 ml HOAc and 15 g KCl. After KCl dissolves, add 15

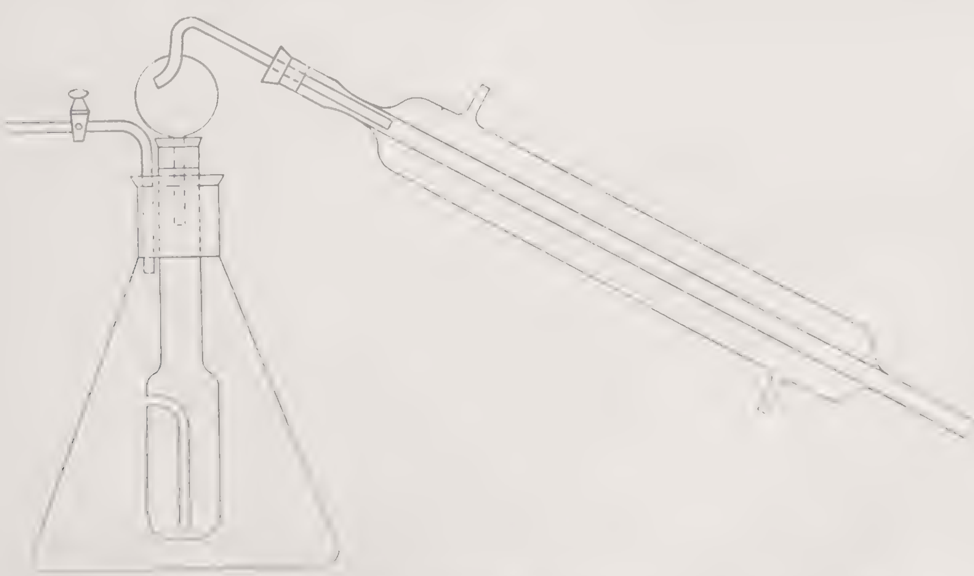

FIG. 22.—APPARATUS FOR DETERMINATION OF VOLATILE ACIDITY

ml alcohol, stir vigorously until $KHC_4H_4O_6$ begins to ppt, and hold in icebox at 15–18° at least 15 hr.

Decant onto gooch prepd with very thin film of asbestos, or onto filter paper in büchner. Wash ppt from beaker with filtrate (keep cold) and finally rinse beaker and filter 3 times with few ml mixt. of 15 g KCl, 20 ml alcohol, and 100 ml H_2O, using not >20 ml wash soln in all. Transfer asbestos or paper and ppt to beaker in which pptn was made; wash gooch or büchner with hot H_2O, using ca 50 ml in all; heat to boiling, and titr. hot soln with 0.1*N* NaOH, using phthln. Increase number of ml 0.1*N* alkali required by 1.5 ml to allow for solubility of ppt. Under these conditions 1 ml 0.1*N* alkali = 0.015 g tartaric acid. To obtain g total tartaric acid/100 ml wine, subtract quantity of tartaric acid added from this result.

11.034 Citric and Malic Acids—First Action

For citric and malic acids occurring in normal wines in small quantities only, use 100 ml sample and evap. to 45 ml. After saponification, **20.044**, proceed as in **20.048, 20.049, 20.060,** or **20.064.**

11.035 Lactic Acid (*7*)—**Official**

Transfer 25 ml sample to 250 ml vol. flask, add ca 25 ml H_2O and 100 ml alcohol, and shake vigorously. Dil. to mark with alcohol and filter thru folded paper. Transfer 200 ml filtrate to 400 ml beaker and evap. to ca 25 ml. Add 50 ml H_2O and again evap. to 25 ml. Transfer material to continuous extractor with 25 ml H_2O and proceed as in **15.012.**

11.036 Tannin—Official—*See* **9.053**

11.037 Crude Protein—Official

Det. N in 50 ml sample as in **2.036** and multiply result by 6.25.

11.038 Pentosans—Official

(Applicable to dry wines only)

Proceed as in **22.049,** except to use 100 ml sample and 43 ml HCl in beginning distn.

Caramel—Official

Mathers Test (*8*)

11.039 REAGENTS

(**a**) *Pectin soln.*—Dissolve 1 g pectin in 75 ml H_2O, add 25 ml alcohol to preserve, and shake well before using.

(**b**) *2,4-Dinitrophenylhydrazine soln.*—Dissolve 1 g 2,4-dinitrophenylhydrazine in 7.5 ml H_2SO_4 and dil. to 75 ml with alcohol. (If kept in g-s. bottle, soln will remain clear and stable several months.)

11.040 PRELIMINARY TEST

Place 10 ml filtered sample in Babcock cream bottle, **15.071(a)**, or other centrifuge tube. Add 1 ml of the pectin soln and mix; add 3–5 drops HCl and mix; fill bottle with alcohol (ca 50 ml), mix, centrifuge, and decant. Dissolve ppt in 10 ml H_2O, and add HCl and alcohol as above; shake well, centrifuge, and decant. Repeat operation until alc. liquid is colorless. Finally, dissolve gelatinous residue in 10 ml hot H_2O. If soln is colorless, caramel is absent; if soln is clear brown, caramel may be present. Confirm as follows: Add 1 ml 2,4-dinitrophenylhydrazine soln, mix, and heat 30 min. in boiling H_2O. Ppt forms if caramel is present.

11.041 CONFIRMATORY TEST

Place 10 ml sample in Babcock cream bottle or suitable centrifuge bottle or tube, ca neutralize with 2% KOH soln, and add 2 ml *5% $ZnCl_2$ soln* and 2 ml 2% KOH soln. Shake thoroly and centrifuge 5–10 min. Decant supernatant carefully from residue and add hot or boiling H_2O. Shake thoroly to wash ppt, centrifuge, and again wash with hot H_2O. Repeat process until upper aq. layer is quite colorless, using any quantity of hot H_2O. To well-washed residue add 50 ml or more *85% alcohol contg 0.5% HCl.* Shake well, centrifuge, and decant upper liquid from any residue that remains. (Caramel is shown by thin brown layer at bottom of bottle.) Add another portion 85% alcohol contg 0.5% HCl, and again centrifuge. Repeat process until upper supernatant is quite clear and colorless.

To facilitate washing, dip centrifuge bottle and contents few min. in beaker of boiling H_2O. After final washing and decanting, dissolve residue in 10 ml H_2O. If soln is clear brown, caramel may be present; confirm with 2,4-dinitrophenylhydrazine as in **11.040.**

Carbon Dioxide—First Action

Manometric Method (*9*)

11.042 REAGENTS

(**a**) *Sodium bicarbonate std solns.*—Dry 150–200 g $NaHCO_3$ over H_2SO_4 24 hr. Weigh designated amounts of dried $NaHCO_3$, transfer to 1 L vol. flasks with ca 700 ml recently boiled H_2O, and add 15 ml NaOH soln, (**c**). Add 200 ml absolute alcohol, mix, cool, and dil. to mark with boiled H_2O. Use 4.2955 g for 225 mg CO_2/100 ml std; 4.7727 g for 250; and 5.2500 g for 275.

(**b**) *Hydrogen peroxide soln.*—10%. Dil. 20 ml 30% H_2O_2 with 40 ml recently boiled H_2O.

(**c**) *Sodium hydroxide soln.*—50%. Transfer 763 g reagent grade NaOH pellets to 1 L Pyrex graduated cylinder, add recently boiled H_2O, cool,

and dil. to 1 L. Mix until soln is complete and set aside at least 5 days until Na_2CO_3 settles, leaving clear soln.

11.043 APPARATUS

(a) *Carbon dioxide apparatus.*—*See* Fig. 23. Vol. of system is ca 350 ml. (Available from New York Laboratory Supply Co., 76–78 Varick St., New York, N. Y., and Berkeley Yeast Laboratory, 3167 College Ave., Berkeley 5, Calif.) Test all glass joints with vac. tester.

(b) *Vacuum tester.*—High frequency self-contained generator operated from 115 volt A.C. outlet. Consists of adjustable interrupter, vibrating spark gap, condenser, resonator coil, and gap tip.

(c) *Magnetic stirrer with Teflon stirring bar.*—Fisher Flexa-Mix or equiv. with stirring bars 1–$1\frac{3}{8}''$ long.

(d) *Vacuum pump.*—Welch Dist-O-Pump or equiv., with motor, single stage, vented exhaust; to be operated with vented exhaust valve open for pumping condensable vapors. Insert 3-way stopcock between pump and app. for allowing air to enter system. Ordinary high vac. pump can be used if H_2SO_4 trap with 3-way stopcock is inserted between pump and app. Change acid frequently.

(e) *Silicone grease, high vacuum type.*—Stable to heat and contains no carbon-to-carbon linkages. Grease may be removed from glassware with Varsol or hot kerosene.

11.044 CALIBRATION OF VACUUM SYSTEM

Pipet 50 ml std $NaHCO_3$ soln and 3 ml 10% H_2O_2 soln into reaction flask, and carefully grease joints. Start magnetic stirrer and evacuate system ca 1 min. Close system to pump at 3-way stopcock, gently tap Hg columns, and read manometer to nearest 0.5 mm to obtain initial reading. Hg levels should remain constant; changes indicate leak, probably caused by insufficient grease at joints.

Add 10 ml H_3PO_4 and continue rapid stirring 5 min. Gently tap Hg columns and read total pressure in cm Hg to nearest 0.5 mm to obtain final reading. Record gas temp. in °C.

Open 3-way stopcock on app. to pump. Then slowly open 3-way stopcock between pump and app. to let air flow into system. Disconnect app. and thoroly wash inner portion of acid dispensing unit and reaction flask. Rinse with acetone and dry with suction.

Det. total pressure from each std soln of $NaHCO_3$ in triplicate and calc. av. vol. of system as follows:

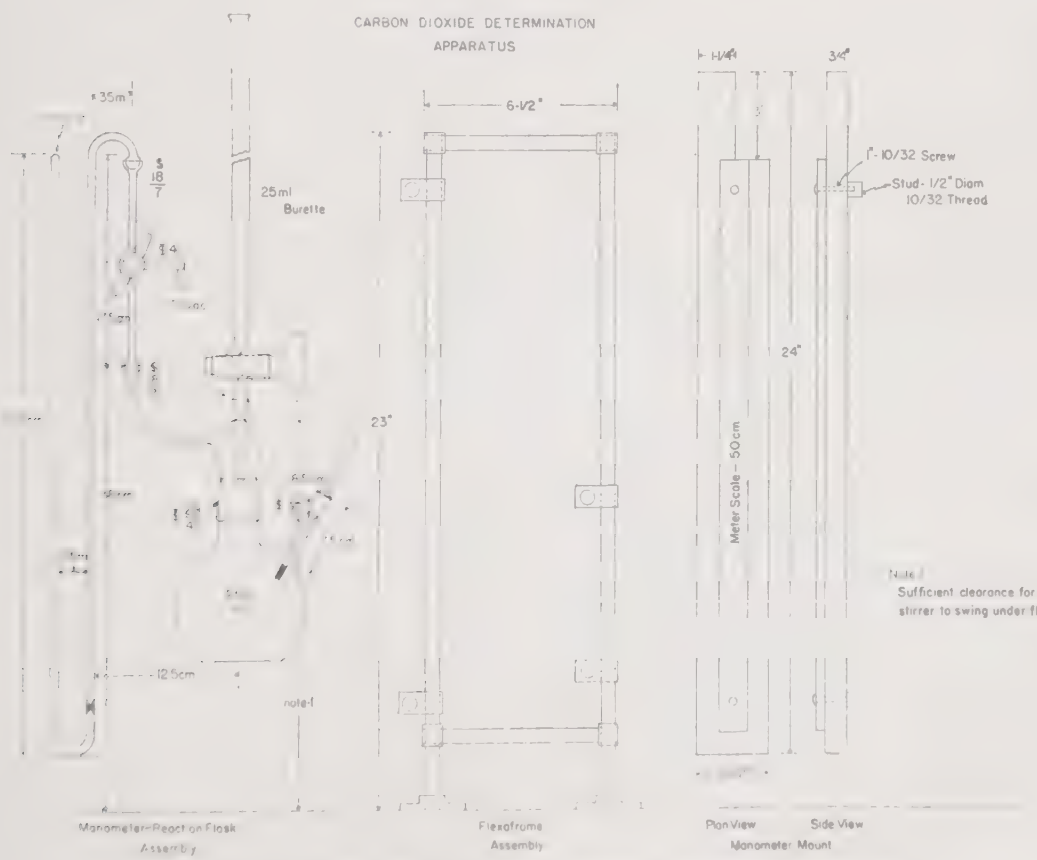

FIG. 23. CARBON DIOXIDE APPARATUS, MANOMETRIC METHOD

From final pressure reading in cm Hg, subtract initial reading and vapor pressure increase due to H_3PO_4 effect as given in table:

% ALCOHOL	VAPOR PRESSURE, CM, INCREASE DUE TO H_3PO_4
0	0.67
5	0.68
10	0.69
15	0.75
20	0.77
25	0.77
50	1.00
75	1.53
100	2.80

Then $V = 76RTg/MP$, where V is vol. of system in L; R is gas constant in L-atmosphere/degree/mole, 0.08205; T is absolute temp., 273 +room temp. in °C; g is wt in g of CO_2 in 50 ml sample; M is molecular wt of CO_2 in g; and P is corrected pressure of CO_2 in cm Hg.

Calc. correction for Hg displaced in manometer tubing, $V_m = \pi r^2 L/2$, where L is difference in height of Hg column in cm; and r is radius of manometer tubing.

Calibrated vol. of system, $V_o = V - V_m$.

In calcg wt CO_2 in sample, Hg displaced in manometer tubing, V_m, is added to calibrated vol. of system, V_o. ($V = V_o + V_m$)

11.045 PREPARATION OF SAMPLE

Chill unopened bottle of wine in ice-salt bath to slightly <32°F (30 min. for 1/10 gallon bottle and 1 hr for 1/5). Open bottle and rapidly add 1.5 ml 50% NaOH soln for each 100 ml wine. Quickly close bottle with rubber stopper, remove from bath, and shake several min. Let contents come to room temp.

11.046 DETERMINATION

Pipet 50 ml sample and 3 ml 10% H_2O_2 into reaction flask, carefully grease joints, and proceed as in **11.044**.

From total pressure in cm Hg, subtract vapor pressure of alcohol-H_2O and pressure due to H_3PO_4 effect. Calc. g CO_2/100 ml wine = $14.327PV/T$.

Volumetric Method (10)

11.047 REAGENTS

(**a**) *Sodium hydroxide std soln.*—0.25*N*. Prep. from NaOH soln (1+1), **42.031(b)** which has stood at least 48 hr. Stdze as in **42.033** or **42.034**, using phthln-thymolphthalein indicator, (**e**). Restdze daily against std HCl, (**b**), in presence of 5 ml $BaCl_2$ soln, (**c**), and indicator, (**e**).

(**b**) *Hydrochloric acid std soln.*—0.25*N*. Stdze against std NaOH, (**a**), using indicator (**e**).

(**c**) *Barium chloride soln.*—Dissolve 60–65 g $BaCl_2.2H_2O$ in 1 L H_2O and neutralize to phthln.

(**d**) *Acid phosphate soln.*—Dissolve 20 g $NaH_2PO_4.H_2O$ in H_2O, add 3 ml H_3PO_4, and dil. to 100 ml.

(**e**) *Phenolphthalein-thymolphthalein mixed indicator.*—Dissolve 1 g phthln and 0.5 g thymolphthalein in 100 ml alcohol.

11.048 APPARATUS

See Fig. 24. Connect 500 ml special distg flask (rubber stopper and ordinary distg flask may be used) thru ca 8 mm glass tubing to series of 3 Pyrex test tubes, 25×200 mm, each fitted at inlet with gas dispersion tube with 12 mm fritted end of coarse porosity and 8 mm stem (Fisher No. 11-138 or equiv.). Connect final exit tube to trapped vac. line or filter pump.

11.049 DETERMINATION

Connect app. and place test tube receivers in beaker of H_2O at <27°. Pipet 20 ml std 0.25*N* NaOH into first 2 receivers and 10 ml 0.25*N* NaOH and 10 ml $BaCl_2$ soln into third.

Pipet 50 ml alk. wine, **11.045**, into distg flask and add 3 ml 10% H_2O_2, **11.042(b)**. Add boiling chips (not marble). Attach vac. line to last receiver and slowly increase vac. until bubbling practically stops; then open vac. line fully. (This keeps system under partial vac. so that stoppers will not be blown out on heating by sudden surge of steam or CO_2.) Add ca 35 ml acid phosphate soln to dropping funnel and carefully admit ca 30 ml into distg flask. Agitate flask gently to mix acid and sample.

Heat gently and when CO_2 evolution slows, heat vigorously. After few ml of liquid distills and top of first receiver is warm, all CO_2 will have been driven into receivers. Close vac. line between trap and receivers and slowly admit air thru dropping funnel until pressure equilibrium is reached.

Transfer contents and rinsings of first 2 receivers and dispersion tubes into titrn flask. (Also add contents of third if $BaCO_3$ has pptd.) Add 50 ml $BaCl_2$ soln and titr. with std HCl to phthln end point.

Wt CO_2 in g/100 ml = [(ml NaOH × normality) − (ml HCl × normality)] × 0.022 × (100/50) × 1.015.

11.050 Sulfurous Acid (*11*)—Official

Proceed as in **27.078**, using 100–300 ml sample contg not >40 mg SO_2. Sweep out flask thoroly with CO_2 or N, stop flow of gas, and add sample to flask thru dropping funnel. Add enough S-free H_2O to make total vol. 300 ml, and then add 20 ml HCl. Let mixt. stand few min. until fumes settle. Adjust burner so that vapors rise no higher than to 1/10 length of H_2O jacket of condenser

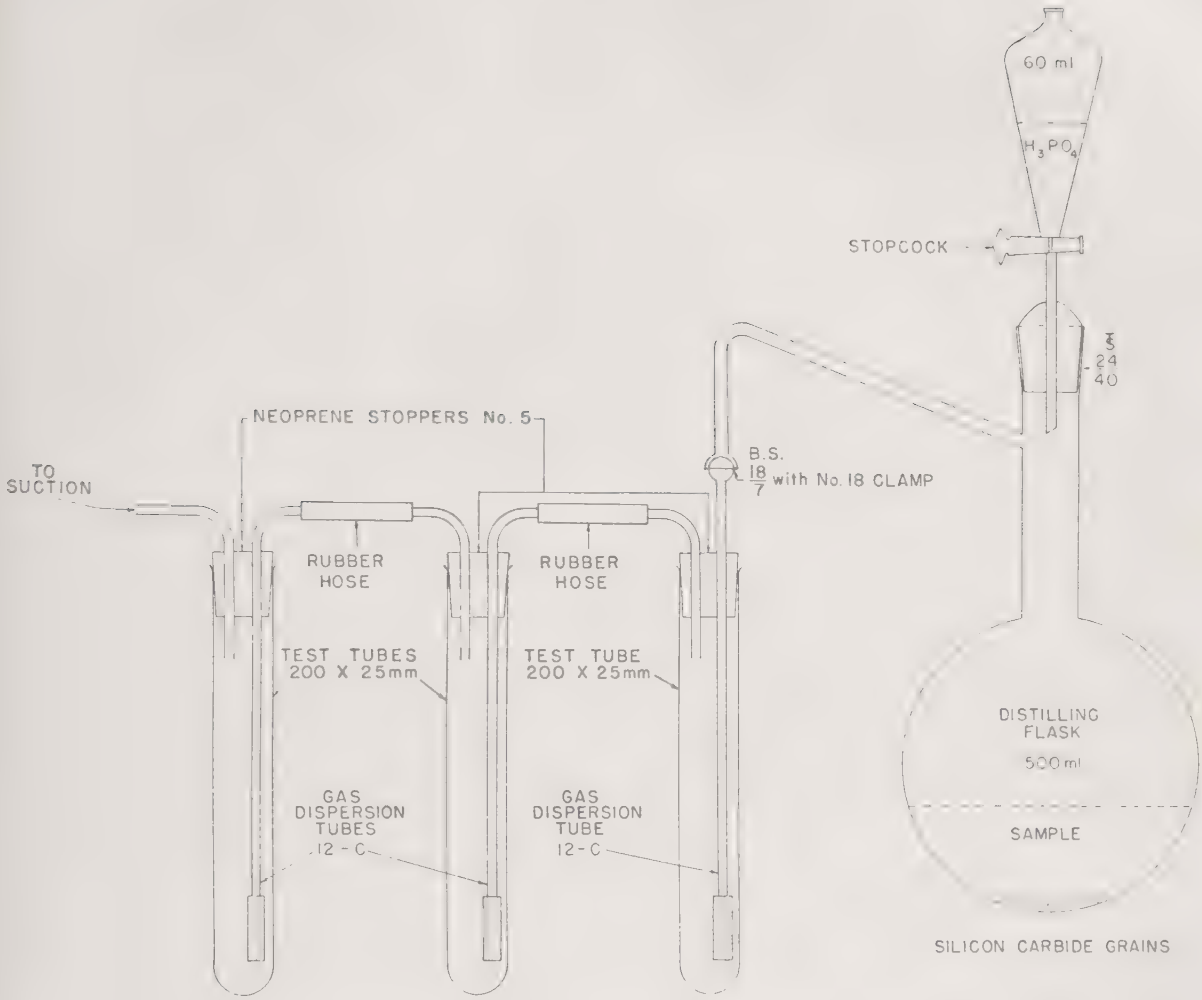

FIG. 24. CARBON DIOXIDE APPARATUS. VOLUMETRIC METHOD

and boil sample 90 min. Adjust flow of gas so that slow, steady stream passes thru receiver during distn. Complete detn as in **27.078.** Report results as mg SO_2/L. (As SO_2 in wine is unstable give sample no preparatory degassing treatment and expose to air for minimum time prior to detn.)

11.051 **Preservatives—Official—***See* Chap. **27**

SELECTED REFERENCES

(*1*) Univ. of Calif. Agr. Expt. Sta. Bull. **651,** 150(1941).
(*2*) J. Assoc. Offic. Agr. Chemists **33,** 1016 (1950).
(*3*) Ibid. **35,** 257(1952).
(*4*) J. Ind. Eng. Chem. **1,** 31(1909); J. Assoc. Offic. Agr. Chemists **22,** 210(1939).
(*5*) J. Assoc. Offic. Agr. Chemists **23,** 183 (1940).
(*6*) U. S. Dept. Agr. Bur. Chem. Bull. **162,** p. 72.
(*7*) J. Assoc. Offic. Agr. Chemists **20,** 605 (1937).
(*8*) Ibid. **31,** 178(1948).
(*9*) Ibid. **42,** 679(1959).
(*10*) Ibid. **43,** 652(1960).
(*11*) Ibid. **23,** 189(1940); **25,** 70, 82, 296(1942); **27,** 85(1944).

12. Cacao Bean and Its Products

12.001 Preparation of Sample—Procedure

(a) Mix powd. products thoroly and preserve in tightly stoppered bottles. Chill sweet or bitter chocolate until hard, and grate or shave to fine granular condition. Mix thoroly and preserve in tightly stoppered bottle in cool place.

(b) Melt bitter, sweet, or milk chocolate by placing in glass or metal container and partly immersing container in bath at ca 50°. Stir frequently until sample melts and reaches temp. of 45–50°. Remove from bath, stir thoroly, and while still liquid, remove portion for analysis, using glass or metal tube, 4–10 mm diam., provided with close-fitting plunger to expel sample from tube.

12.002 Moisture (*1*)—First Action

Dry 2 g prepd sample, **12.001,** to constant wt in Pt dish in air oven at 100°. (Al dish may be used when ash is not detd on same sample.) Report loss in wt as H_2O.

12.003 Ash—Official

Proceed as in **29.012** or **29.013,** using enough sample to contain ca 1 g H_2O-, sugar-, and fat-free material.

12.004 Soluble and Insoluble Ash—Official

Proceed as in **29.015,** using ash from **12.003.**

12.005 Alkalinity of Soluble Ash—Official

Proceed as in **29.016,** using filtrate from **12.004.**

12.006 Alkalinity of Insoluble Ash—Official

Proceed as in **29.017,** using insol. ash obtained in **12.004.**

12.007 Ash Insoluble in Acid—Official

Proceed as in **28.005,** using total ash obtained in **12.003,** or H_2O-insol. residue obtained in **12.004.**

12.008 Total Nitrogen—Official—*See* 2.036

SHELL (*2*)

In Cacao Nibs—Official

12.009 TRIER FOR SAMPLING

Use double-tube, separate-compartment grain trier to collect samples of nibs from bins, trucks, and sacks. Tubes of trier are of No. 16 B & S gauge (0.0508″) seamless metal. Outer tube is $1\frac{3}{8}$″ o.d., and outer and inner tubes fit each other closely. Width of openings in outer tube is 15/16″, and in inner tube 1″. Length of such openings in both tubes is $3\frac{1}{2}$″, except that length of opening of compartment nearest point of trier may be 3–$3\frac{1}{2}$″. Each compartment coincides with and is of same length as its opening in inner tube. Openings of inner and outer tubes match when trier is open for sampling. Distance between adjacent compartments is $1\frac{1}{2}$–2″, and distance between point of trier and compartment end nearest point is not $>1\frac{1}{8}$″.

12.010 COLLECTION OF SAMPLE

(a) *From bins or trucks.*—Collect ca 10 lb sample by probing with trier, **12.009.** Probe nibs to floor of bin or truck, spacing individual probings ca equidistant from each other thruout top area of nibs. If contents of bin are inaccessible, or depth is greater than length of trier from its point to 2″ above compartment end nearest handle, take sample from chute thru which bin is being filled or emptied as in (c).

(b) *From sacks.*—Collect ca 10 lb sample by probing with trier, **12.009.** Length of trier from its point to 2″ above compartment end nearest handle equals or exceeds depth to which sacks are filled. Probe with trier thru entire depth of nibs in sack. Probe number of sacks equal to at least square root of total number of sacks in lot. If lot is <12 sacks, probe at least $\frac{2}{3}$ of them; if 12 or more, probe at least 8.

(c) *From chutes.*—Collect ca 10 lb sample by catching momentarily and at regular intervals, in suitable receptacle, cross section of stream of nibs from chute. Continue sampling throughout time lot of nibs being sampled is passing thru chute.

12.011 REDUCTION OF SAMPLE

Using sample divider of type described in U. S. Dept. Agr. Bull. No. 287, September 14, 1915, and No. 857, June 25, 1920, reduce size of sample collected, **12.010,** to ca $\frac{1}{2}$ lb. Weigh reduced sample to nearest 0.05 g.

12.012 DIVISION OF SAMPLE

(a) *Hand division.*—Screen reduced sample, **12.011,** in successive portions of 75–100 g, on circular ca 8″ diam. No. 10 sieve. Collect material remaining on sieve and designate as *L*. Screen material that passed thru sieve on another circu-

lar No. 20 sieve 6 or 8″ diam. Collect portion remaining on sieve and designate as S. Collect material passing thru sieve and designate as F. Treat portions L, S, and F, resp., as in **12.013**(a), (b), and (c).

(b) *Machine division.*—Use sample-size, grain-cleaning mill of type described on p. 16 of U. S. Dept. Agr. Farmers' Bull. 1747 (1935). (The "Clipper Office Tester" grain, seed, and bean tester manufactured by A. T. Ferrell and Co., Saginaw, Mich. is satisfactory). Fit into lower slot of mill single screen with circular openings 0.083–0.093″ diam. Machine is provided with settling traps to catch all material blown out by fan. Inclined slide under screen is provided with removable slat in such position that, when it is removed, material passing thru screen is discharged from mill without going to fanning chamber. Remove this slat, start mill, and slowly pour reduced sample, **12.011**, over upper part of screen. Designate as L' material that does not pass thru screen and is not removed by fanning. Collect material that passes thru screen and screen again thru No. 20 sieve, (a). Collect portion remaining on sieve and designate as S. Collect portion passing thru sieve and designate as F. Reserve F for treatment as in **12.013**(c).

Replace slat in mill and, without removing L' or fannings, start mill and pour S slowly onto upper part of screen. Let material thus cleaned combine with L', and designate combination as $L'S'$. Treat $L'S'$ as in **12.013**(a). Remove combined fannings from settling traps, and screen on No. 10 sieve, (a). Collect portion remaining on sieve and designate as LS. Collect portion passing thru sieve and designate as SS. Treat LS as in **12.013**(a) and SS as in **12.013**(b).

12.013 DETERMINATION

(a) Place L (from hand division), or $L'S'$ (from machine division), on large sheet of sized paper. Scatter 2–3 g L or 4–5 g $L'S'$ over area of paper 3-4″ diam. Examine scattered portion and remove pieces of shell with spatula or tweezers. Examine entire portion of L or $L'S'$ progressively in this manner. Sep. shell from LS (from machine division) in same manner. Reserve sepd shell for later combination with shell from other fractions.

(b) If S (from hand division) weighs >4.5 g and appears to contain large quantity of shell, weigh it accurately to nearest 10 mg. Mix entire portion by pouring gradually several times from one glazed paper to another, each time forming conical pile; flatten and quarter last pile formed, and combine alternate quarters. If necessary, mix and again reduce by quartering to obtain 3–4.5 g, and weigh accurately fraction thus obtained to nearest 10 mg. If quantity of shell in S appears to be small, use entire portion. Sep. shell from S or fraction thereof and from SS (from machine division) as follows:

Place blotting paper ca 19×24″ on firm supporting plane inclined at 21–24° from horizontal. Pour all material gradually and in successive portions from elevation of 2–3″ along upper end of blotter. Shake blotter slowly parallel to plane to cause nib material to roll down, and at intervals remove material collected at bottom. Toward end of procedure shake blotter more rapidly to detach most of nib material. After removing shell adhering to blotter, repeat procedure on last portions of material collected at bottom of blotter. Using reading glass, complete sepn of shell and nibs with spatula or tweezers by examining portions until all material is examined. Except in sepns from a fraction of S, reserve sepd shell for combination with that obtained from other sepns. In case of sepns from a fraction of S, weigh sepd shell to nearest mg and calc. total wt shell in S.

(c) Place F (obtained from either hand division or machine division) in 400 ml beaker ca half full of alcohol-CCl_4 mixt. $(1+2\frac{1}{8})$. (Mixt. should have sp. gr. of 1.335–1.345 at temp. used as compared to H_2O at 20°.) Stir ca 1 min., slowly at the last, and let stand 3–4 min. Skim off floating nibs with tea strainer made with ca No. 40 wire cloth. Decant liquid and any suspended material from beaker without disturbing residue until 2–4 ml remains. Wipe inside of beaker above liquid with filter paper, moistened in the alcohol-CCl_4 mixt., to remove all nib material. Add ca 25 ml petr. ether, swirl liquid in beaker few times, let residue of shell settle, and carefully decant liquid. Let remaining liquid evap. and dry shell on steam bath. Reserve shell for combination with other fractions of shell.

(d) Combine all shell obtained from L, S, and F (from hand division), or $L'S'$, LS, SS, and F (from machine division) and weigh combined shell from reduced sample. If fraction of S was used, combine L and F, weigh, and add calcd wt shell in S to obtain wt shell from reduced sample. Report result as % by wt of shell in nibs.

In Cacao Products Other Than Cacao Nibs

(Following methods include detns the results of which can be used to estimate amount of shell when compared with corresponding values obtained on authentic samples of cacao shell.)

12.014 Crude Fiber (3)—Official

(a) *In cacao products not containing dairy ingredients.*—Treat 7 g liquor (or quantity of sweet chocolate or cocoa equiv. to 7 g liquor) in centrifuge bottle with two 100 ml portions ether, centrifuging and decanting supernatant after each

addn. Dry residue in oven at ca 100° and then powder in bottle with flat-end glass rod. If necessary, grind material in mortar and ext. third time with ether. Wash mixt. in bottle with three 100 ml portions H_2O at room temp., shaking well each time, until no cacao material adheres to bottle. Centrifuge 10–15 min. after each washing, and decant aq. layer. Wash residue in same fashion with two 100 ml portions alcohol and one 100 ml portion ether. Transfer residue to Pt dish, dry to constant wt, and grind in mortar. Weigh 2 g dried material and det. % crude fiber (D) as in **22.040**, using linen for both acid and alk. filtrations. Calc. % crude fiber on H_2O-, fat-, and sugar-free basis (E) by formula $E=0.7D$.

(b) *In cacao products containing dairy ingredients.*—Treat 50 g milk chocolate with three 100 ml portions ether in centrifuge bottle, centrifuging and decanting supernatant after each addn. Dry residue in bottle and powder with flat-end glass rod. Shake with 100 ml *1% $Na_2C_2O_4$ soln,* and let stand 30 min. Centrifuge and decant supernatant. Wash in bottle with three 100 ml portions H_2O at room temp., shaking well each time, until no cacao material adheres to bottle. Centrifuge 10–15 min. after each washing and decant aq. layer. Wash residue in same fashion with two 100 ml portions alcohol and one 100 ml portion ether. Transfer residue to Pt dish, dry to constant wt at 100°, and grind in mortar. Weigh 2 g of the dried material and det. % crude fiber as in **22.040**, using linen for both acid and alk. filtrations. % crude fiber found $\times 0.7=$ % crude fiber on fat-, sugar-, H_2O-, and milk-free basis.

12.015 Pectic Acid (*4*)—Official

(Sweet chocolate, usually characterized by its color, may contain small quantities of milk solids. When in doubt, use method for milk chocolate, **12.015(c)**.)

(a) *In sweet chocolate containing no milk solids.*—(1) *Extraction of fat.*—Weigh, within ± 0.15 g, quantity (14–60 g) of well-mixed grated sample contg 4.7–5.2 g dry, fat-free cacao, and place in one or two 250 ml centrifuge bottles. (If sample is >50 g, distribute it ca equally between 2 bottles.) (Make detns in duplicate.) Add 120 ml petr. ether (b. p. 30–65°), or ether, at ca 30°, to each bottle, shake thoroly, centrifuge, and decant supernatant. Repeat extn with another 100 ml solvent; then ext. with 100 ml alcohol, decant, and discard exts.

(2) *Extraction of color, tannins, etc.*—Add to each bottle (from graduate) 150 ml *acidified 82% alcohol* (10 ml HCl+432 ml alcohol dild to 500 ml with H_2O) that has been warmed so that temp. of liquid in centrifuge bottle is 55°. Stopper, shake vigorously 2 min., centrifuge 6–8 min., decant, and discard supernatant. Add 100 ml alcohol to residue in each bottle, shake, centrifuge as before, decant, and discard exts.

(3) *Extraction of pectin.*—Measure 150 ml H_2O in graduate, add ca 75 ml to 1 bottle, stopper, shake vigorously to disperse residue thoroly, decant into other bottle contg remainder of sample, and again shake vigorously until residue is thoroly dispersed. Decant mixt. into 500 ml wide-mouth erlenmeyer, rinse mouth of bottle with ca 1 ml H_2O from wash bottle, and complete transfer of residue from bottles with ca 45 and 30 ml successive portions of H_2O remaining in graduate. Make mixt. in flask just alk. to litmus with $NH_4OH(1+1)$ (ca 0.7 ml; note quantity used; *See* NOTE). Acidify with HOAc, add 0.5 ml excess, and then add 50 ml 2% $(NH_4)_2C_2O_4.H_2O$ soln, using soln to wash down sides of flask.

Pass glass stirrer, with 1–1¼″ diam. loop (perpendicular to shaft on end), loosely thru hole in rubber stopper or thru glass tube of slightly larger diam. held in rubber stopper placed in mouth of flask. Attach shaft of stirrer to motor, or air rotor, to stir contents of flask continuously, immerse flask below level of contents in H_2O bath held at 90–92°, and stir moderately 3 hr. If level of liquid in flask is appreciably reduced, add enough hot H_2O to bring back to original level.

Remove flask, cool to 45°, transfer contents quantitatively to 250 ml vol. flask, dil. to vol. with H_2O at 45°, and add 1.5 ml excess to correct for vol. of cacao solids. Mix contents well, pour into centrifuge bottle, and centrifuge at 1800 rpm ca 15 min. Decant supernatant ext., which may be turbid or opalescent, into 400 ml beaker. Rinse any residue in flask into centrifuge bottle with alcohol and reserve this cacao residue for further treatment to estimate fat-free cacao in sample. Warm ext. to 45°, pour into graduate, note vol., and return to beaker. Rinse graduate with two 5 ml portions H_2O, and add to beaker. Cool in bath to 15–17°, make alk. to phthln (internal indicator) with 15% NaOH soln, and add 11 ml excess. (*See* NOTE.) Stir, and let stand in bath 20 min. at 15–17°. Decant alk. liquid into two 250 ml centrifuge bottles, distributing vol. ca equally. Let drain, and rinse twice with 5–8 ml cold H_2O, adding 1 rinsing to each bottle. Add to each bottle, with stirring, 10 ml HCl, and then add gradually, with continued stirring, 40 ml alcohol. Add to each bottle 0.8–1.0 g mixt. of Filter-Cel and Celite 545 (1+1). Stir, rinse rod, stopper bottles, shake well, and centrifuge 10–12 min. Decant and discard supernatants without disturbing sediment, and wash residues once by shaking contents of each bottle with 100 ml alcohol, centrifuging, and decanting supernatants.

Add 75 ml H_2O to 1 bottle, stopper, and shake well. Make slightly alk. with few drops NH_4OH (1+1) and shake again. Decant liquid into second

bottle, stopper, shake again, make alk. to litmus with NH_4OH (1+1), and add 0.5 ml in excess. Stopper, and shake thoroly 1–1.5 min. to dissolve pectic acid ppt. (Drops of liquid clinging to lip of bottle may be washed into second bottle with small squirt of H_2O from wash bottle; otherwise do not rinse at this point.) Filter, with suction, thru hardened paper (Whatman No. 41-H or 54 or equiv.) on 11 cm büchner. Let bottle drain well; then rinse bottle twice with 25 ml portions H_2O, each contg 1–2 drops NH_4OH (1+1), pour rinsings on filter, and wait for each rinse to drain thru filter before adding another.

Decant filtrate into 250 ml centrifuge bottle, let flask drain, and rinse twice with 5 ml H_2O. (Use of bell jar permits filtration directly into centrifuge bottle.) Add 5 ml HCl to contents of centrifuge bottle, stir in 90–100 ml alcohol, rinse rod with alcohol (do not add filter-aid), stopper, shake, and centrifuge 8 min. at 1500–1800 rpm. Decant supernatant into beaker, retaining most of ppt in bottle, and filter liquid thru 15 cm Whatman No. 41-H paper (or equiv.) on fluted funnel. Pour ppt and liquid remaining in centrifuge bottle onto filter paper and drain thoroly. (Do not rinse.)

Quantitatively transfer ppt in bottle and on filter to 250 ml beaker, using total of 75 ml 60–75° H_2O. Cool beaker and contents in bath at 15–17° and add, with stirring, 15% NaOH soln (also cooled) until mixt. is alk. to phthln (internal indicator). Add 3 ml excess and let mixt. stand in bath 15 min. at 15–17°. During this time, heat on steam bath 2 wash bottles, contg, resp., *wash solns: A*, mixt. of 200 ml H_2O, 50 ml alcohol, and 20 ml HCl (1+2.5); *B*, 400 ml alcohol dild to 950 ml with H_2O.

Remove beaker from bath, acidify contents with 10 ml HCl (1+2.5) while stirring, and dil. to 100 ml with H_2O. (Estimate vol. by comparison with 100 ml in similar beaker.) Add few glass beads, cover beaker, bring contents to boil, and boil 5 min. Remove from heat, add, with stirring, 10 ml HCl, and then add 400 mg *prepd asbestos* (previously alkali- and acid-washed and ignited, and free of coarse particles). Stir 40 sec., and immediately filter thru Whatman No. 41-H paper (or equiv.) on 7–11 cm büchner with very gentle suction. (Suction should be so gentle that it can hardly be felt when thumb is placed on rubber tube before attaching tube to flask; sample should filter in small steady stream, and filtrate should be clear or only slightly opalescent, with no immediate sepn of ppt.) Wash beaker and filter with three ca 25 ml portions wash soln *A*, and then with four or five ca 25 ml portions wash soln *B* to remove acid. (Washings should be clear and pass thru filter readily. Ignore any appearance of ppt in flask at this stage.)

Place filter and ppt on fairly large, short-stem funnel, and wash pectic acid ppt and asbestos into Pt dish with hot H_2O. Det. blank on 400 mg asbestos by adding it to hot acid soln, filtering, and drying in same manner as sample. Heat dishes on steam bath until asbestos and ppt appear thoroly dry. Dry sample and blank in oven at 100° to constant wt (±0.2 mg; ca 1 hr), cool in desiccator, weigh, ignite, cool, and reweigh. Loss in wt sample − loss in wt blank = wt pectic acid in aliquot taken.

This wt × 250/vol. ext. taken

= wt pectic acid in sample.

To obtain dry, fat-free cacao in sample, add 100 ml alcohol to cacao residue reserved in centrifuge bottle, stopper bottle, shake well, centrifuge, and decant alcohol. Again shake with 100 ml alcohol, rinse stopper, and wash down sides of bottle with alcohol from wash bottle; centrifuge and decant. Repeat extn, using 100 ml ether, washing down sides, centrifuging, and decanting. Let residual ether evap. Using brush and spatula, quantitatively transfer residue to tared Al dish with cover; dry dish and contents 1–2 hr in oven at 100°; cover dish, cool in desiccator, and weigh. Wt residue × 1.9 = wt dry, fat-free cacao in sample.

Wt pectic acid × 100/wt dry, fat-free cacao

= % pectic acid.

(b) *In chocolate liquor, breakfast cocoa, cocoa, and low-fat cocoa.*—Place ca 15 g cocoa or 25 g chocolate liquor, prepd as in **12.001**, in centrifuge bottle. To remove most of fat, shake contents of bottle thoroly with 100 ml petr. ether (b. p. 30–65°) or ether; centrifuge, decant supernatant, and repeat extn with another 100 ml petr. ether or ether. Shake residue with third portion solvent, and filter thru Whatman No. 41-H or 54 paper (or equiv.) on 11 cm büchner with gentle to moderate suction. (Apply vac. and wet filter with solvent before starting filtration.) Let residue suck dry, transfer it to porcelain dish or casserole, grind gently with pestle to pulverize and mix it, and transfer to Al dish with cover. Dry ca 45 min. in oven at 100°, cover dish, and cool in desiccator. Weigh 5 g of the dry, fat-free residue into 250 ml centrifuge bottle, and proceed as in (a)(1), last sentence, beginning "then ext. with 100 ml alcohol . . ." and continue as in (2) and (3) thru next-to-last par. (directions for calcg wt pectic acid in sample).

(Wt pectic acid found/5) × 100

= % pectic acid in dry, fat-free cacao.

(No estimation of dry, fat-free cacao is necessary, since weighed amount of dry, fat-free cacao is used for the pectic acid detn.)

(c) *In products containing milk solids.*—(1) *Removal of fat.*—Weigh (±0.2 g) sample contg

ca 5 g dry, fat-free liquor (60–110 g milk chocolate, etc.), and distribute ca equally between two 250 ml centrifuge bottles. Add 120 ml petr. ether or ether at 25–30°, shake thoroly, centrifuge, and decant supernatant. Add another 120 ml portion tepid solvent to each bottle, shake thoroly, centrifuge, and decant supernatant. In same manner ext. contents of each bottle with 100–110 ml acetone.

(2) *Extraction of milk protein.*—Add enough acetone (ca 90 ml) to make total of ca 110 ml with acetone remaining in residue. (Estimate on basis that ½ original sample of 75 g retains ca 20 ml acetone in residue of each bottle.) Stopper, and shake vigorously to disperse residue thoroly. Quickly add to each bottle 100 ml *triethanolamine soln* (90 ml triethanolamine dild to 500 ml with H_2O), stopper immediately, and shake well 2 min. Let stand ca 1 min. for foam to rise; then centrifuge 12–14 min. at 1500–1800 rpm. Carefully decant and discard supernatant without disturbing residue, and ext. residue with 100–120 ml mixt. of acetone and the triethanolamine soln (110+100), centrifuging and decanting as before. Then add 100 ml 85% alcohol to each bottle, shake, centrifuge, decant, and discard ext.

Add to residue in each bottle 15–20 ml of the acidified 82% alcohol, (**a**)(2), stir, and add enough HCl to make residue acid to litmus. Continue as for sweet chocolate, beginning with (**a**)(2).

Note: Quantity of NH_4OH used should be noted, excess avoided, and approx. concn of NH_3 detd. (Soln becomes much less concd on standing from loss of NH_3 around stopper.) This is necessary because in first hydrolysis (saponification) of pectin, part of NaOH soln added (after soln of sample has been made alk. to phthln) is used up in replacing with Na the NH_4 in NH_4 salts present. The 11 ml 15% NaOH soln added furnishes excess of 4–5 ml of this soln over quantity required to neutralize acid and replace NH_4 with Na, provided not >1.15 ml 7.5*N* NH_4OH (=2.3 ml 15% NaOH soln) is added to neutralize residual HCl. (Ca 3.75 ml 15% NaOH soln is needed to replace with Na the NH_4 in $(NH_4)_2C_2O_4$ soln used in extn of pectin.) If >1.15 ml of the NH_4OH soln is needed to neutralize HCl in (**a**)(3), correspondingly increase quantity of 15% NaOH soln used for saponification, but avoid excess of >5–6 ml.

12.016 Ash Insoluble in Acid—Official—*See* 12.007

CACAO PRODUCTS PROCESSED WITH ALKALIES

12.017 Ash—Official—*See* 12.003

12.018 Soluble and Insoluble Ash—Official—*See* 12.004

12.019 Alkalinity of Soluble Ash—Official—*See* 12.005

12.020 Alkalinity of Insoluble Ash—Official—*See* 12.006

12.021 CHOCOLATE LIQUOR (*5*)—FIRST ACTION

Ext. 25–50 g sample (50 g if light color, indicating low liquor) as in **12.014,** except to use in first aq. extn 200 ml H_2O for products referred to in (**a**) and 200 ml *1% $Na_2C_2O_4$ soln* for products referred to in (**b**). Follow method thru aq., alcohol, and ether extns only.

With aid of small portions of ether (45, 20, 15 ml, etc.) transfer residue resulting from ether, alcohol, and aq. extns to tared Al dish provided with tight-fit cover. Use small quantity of acetone and policeman to transfer any material that sticks to bottle. Evap. liquid carefully on steam bath or hot plate, and dry residue in oven at 100°. Cover dish, cool in desiccator, and weigh.

To obtain wt dry, fat-free cacao mass, multiply wt residue by factor 1.43. To obtain wt chocolate liquor multiply wt dry, fat-free cacao mass by factor 2.2. (This factor is based on fat content of 54% in chocolate liquors.)

FAT

Quantitative Determination

12.022 *Method I.* (*6*)—*Official*

(Not applicable to cacao products contg milk ingredients or to products prepd by cooking with sugar and water, and drying.)

Prep. in Knorr extn tube, **16.017**(**d**), first par., 6 mm tightly packed mat of asbestos purified as for detn of crude fiber, **22.038**(**c**), and carefully freed from coarse pieces. (Allihn type filter tube with coarse fritted disk such as Ace Glass Inc. No. 8571 is also satisfactory.) Wash filter with alcohol, ether, and little petr. ether. (*All* petr. ether used in this detn must be redistd at <60°.) Weigh 2–3 g prepd sample, **12.001,** into tube and insert tube into rubber stopper in filtering bell jar connected to suction thru 2-way stopcock, taking care that no rubber particles adhere to tip of stem. Place weighed 200 ml erlenmeyer at such height that tube stem passes thru neck into flask. (Lengthen stem of tube if necessary.) Fill tube to ca ⅔ capacity with redistd petr. ether, and with flat-end rod stir sample thoroly, crushing all lumps. Let stand 1 min. and drain by suction. Regulate suction so that collected solvent in flask will not boil violently. Release vac. after each draining before adding more solvent. Add solvent from wash bottle while turning tube between thumb and finger so that sides of tube are washed down by each addn. Repeat extns, with stirring, until fat is removed (usually 10 extns). Remove tube with stopper from bell jar, wash traces of fat from end of stem with petr. ether, evap. solvent, and dry to constant wt at 100°.

12.023 *Method II. (7)—Official*

(Applicable to cacao products contg milk ingredients or to products prepd by cooking with sugar and water, and drying.)

Weigh accurately, into 400 ml beaker, 10–20 g milk chocolate or 50 g chocolate malted milk or chocolate powders contg milk, and add 30 ml H_2O and 25 ml HCl. Heat 30 min. on steam bath, stirring frequently; add 5 g filter-aid (diatomaceous earth type) and 50 ml ice-H_2O, and chill 30 min. in ice-H_2O. Fit heavy piece of crude-fiber quality cloth, **22.038(c)**, into Coors No. 1A büchner and moisten with H_2O; apply gentle suction and completely overlay cloth with filter-aid (3 g filter-aid suspended in 30 ml H_2O, poured over funnel, and allowed to drain).

Filter hydrolyzed mixt. by gentle suction, rinsing beakers 3 times with ice-cold H_2O (but do not suck pad dry until transfer and washings are complete). Finally, wash 3 times with ice-cold H_2O, tamping tightly with flat-end rod after last washing, and suck dry. Strip linen from cake and transfer cake to original beaker. With small piece of filter paper, transfer to beaker any material adhering to funnel. Wash funnel with petr. ether and add to cake in beaker. Evap. ether on steam bath.

Break up cake with stirring rod and keep on steam bath until contents pulverize easily and appear to be dry. Place in oven 1 hr at 100°. Add 15 g powd. anhyd. Na_2SO_4 and mix well. Transfer this dry mass to large Knorr-type tube (ca 175 ml capacity fitted with ½″ perforated metal disk, over which is laid dry asbestos pad ca ⅜″ thick) or to fritted 150 ml, 60–65 mm, medium porosity büchner fitted with layer of asbestos covered with qual. filter paper and with circle of wire screen (ca 20 mesh) over the paper. Wash beaker with 50 ml petr. ether, pouring rinsings into tube. Ext. material in tube with six 50 ml portions petr. ether. Stir sample thoroly with each 50 ml petr. ether with glass rod, crushing all lumps. Let stand 2 min. and drain by gentle suction into tared 250 ml flask. Evap. petr. ether on steam bath and dry fat to constant wt.

12.024 Separation and Preparation of Fat for Determination of Constants—Procedure

(**a**) *Not applicable to cacao products containing milk ingredients or to products prepared by cooking with sugar and H_2O, and drying.*—Sep. fat from 10–40 g sample (depending upon fat content) by shaking material with two or three 100 ml portions ether. Centrifuge and decant each portion. Combine portions in beaker and evap. most of ether on steam bath. Filter ether exts thru dry, folded paper and dry at 100°.

(**b**) *Applicable to cacao products containing milk ingredients or to products prepared by cooking with sugar and H_2O, and drying.*—Proceed as in **12.023**, using 20 g sample in case of milk chocolate and combining fat obtained in duplicate detns for examination.

12.025 Iodine Absorption Number—Official—*See* **26.017** or **26.019**

12.026 Melting Point—Official

Proceed as in **26.012**. Keep fat at least 24 hr in cool place before making detn.

12.027 Index of Refraction—Official—*See* **26.007** or **26.009**

12.028 Reichert-Meissl and Polenske Values (*8*)—**Official**—*See* **26.027**

12.029 Milk Fat in Milk Chocolate—Official

Estimate quantity of milk fat in milk chocolate from following formula: $C = (AX + BY)/5$; where A = g butter fat in 5 g mixed fat; $B = (5 - A)$ = g cacao fat in 5 g mixed fat; C = Reichert-Meissl number of extd fat; X = Reichert-Meissl number of authentic butter fat; and Y = Reichert-Meissl number of authentic cacao butter.

Then wt butterfat, A, in 5 g mixed fat $= 5(C - Y)/(X - Y)$, and

$\%$ butterfat $= \%$ total fat $\times (C - Y)/(X - Y)$.

12.030 Saponification Number—Official—*See* **26.023**

12.031 Detection of Coconut and Palm Kernel Oils in Cacao Butter and Fat Extracted from Milk Chocolate (*9*)—**First Action**

(**a**) *Examination of cacao butter.*—Saponify 5 g sample with 15 ml *alc. KOH soln* (25 g in 200 ml alcohol) and evap. alcohol on steam bath. Prep. blank on pure cacao butter at same time. Add 5 ml H_2O and again evap. to remove last trace of alcohol. Dissolve soap in 100 ml H_2O, cool to room temp., and add, while stirring, 100 ml satd NaCl soln. Let stand 15 min., stirring occasionally, and then sep. soap by filtration thru büchner. To 100 ml filtrate add, while stirring, 100 ml satd NaCl soln and let stand 15 min. (Only slight ppt should appear.) Filter, add 1 drop phthln to filtrate, neutralize with HCl (1+3), and add 0.5 ml excess. If sample consists of pure cacao butter, acidified soln will remain clear; if coconut or palm kernel oil is present, soln will become turbid or milky.

(**b**) *Examination of fat extracted from milk chocolate* (*10*).—Milk fat, if present in cacao butter subjected to this test, produces turbidity less intense than that produced by same % coconut or palm kernel oil. For example, cacao butter contg 10, 15, or 20% milk fat produces, resp., no

opalescence, faint opalescence, or more pronounced opalescence. For this reason, when fat to be examined has been extd from cacao product that contains lactose or casein, multiply % lactose in cacao product by 0.8, or % casein by 1.1, to obtain % milk fat in product, and from this result calc. % milk fat in total fat. If this corresponds to 15% or less, blank of cacao butter contg 15% milk fat may be used; otherwise make up mixt. of cacao butter and milk fat in proportions indicated by the calcns.

Test fat extd from sample under examination as in (a), but use the prepd mixt. of cacao butter and milk fat instead of pure cacao butter for blank. If fat being tested contains coconut oil or palm kernel oil, last filtrate, when acidified, will be more turbid or milky than blank.

Silver Number for Detection of Coconut and Palm Kernel Oils (*11*)—Official

12.032 REAGENTS

(**a**) *Potassium hydroxide soln.*—Dissolve 750 g KOH in H_2O and dil. to 1 L with H_2O.

(**b**) *Magnesium sulfate soln.*—Dissolve 150 g $MgSO_4.7H_2O$ in H_2O and dil. to 1 L with H_2O.

(**c**) *Sodium nitrate.*—Crystals as Cl-free as practicable (0.002% or less).

(**d**) *Ferric indicator.*—Satd. Use $FeK(SO_4)_2.12H_2O$ or $FeNH_4(SO_4)_2.12H_2O$.

12.033 DETERMINATION

Weigh 10 g fat into 250 ml beaker and add 40 ml alcohol and 5 ml of the KOH soln. Saponify mixt. and evap. to dryness on steam bath. Take up soap in 150 ml H_2O, warming if necessary. Cool, and dil. to 250 ml.

Pipet 200 ml soln into 500 ml erlenmeyer. Close flask with stopper holding thermometer and having small groove lengthwise in side. Place flask in H_2O bath held at ca 80°. When sample reaches ca 80°, loosen stopper, and pipet in 50 ml of the $MgSO_4$ soln. Shake flask with rotary motion. Replace stopper and thermometer and let flask remain in bath 8–10 min. longer at 70–80°, shaking occasionally. Remove flask and cool under tap, with shaking, to 20–25°. Remove stopper and thermometer, stopper tightly, and shake vigorously 4 min. Let flask stand in bath at 20–25° until aq. layer seps at bottom. Filter thru büchner, removing all liquid possible by pressing with glass or plastic spoon. Det. blank on cacao butter in same way.

Neutralize 200 ml filtrate in 250 ml vol. flask with ca 0.5N H_2SO_4 until colorless to phthln. Add 20 g of the $NaNO_3$ crystals, and when dissolved, add 22.5 ml 0.2N $AgNO_3$. Dil. to mark and shake 3 min. Let soln stand short time and filter thru folded paper. To 200 ml filtrate add 6 ml of the ferric indicator and 4 ml HNO_3 (4+3). Titr. with 0.1N NH_4SCN to first color change (reddish brown).

Ag number (mg Ag used/g fat) $= (a-b) \times 2.107$, where $a = 1.6 \times$ ml 0.2N $AgNO_3$ added and $b =$ ml 0.1N NH_4SCN used in back-titrn.

Factor $2.017 = 10.788 \times$ (mg Ag/ml 0.1N soln)/$5.12 \times$ (g fat in aliquot titrd).

Ag number of palm kernel and coconut oils and of stearins varies from ca 26 for stearins to 60 for whole coconut oil. Milk fat gives value of ca 11.6, and cacao butter, 0.6.

Critical Temperature of Dissolution of Fat in Acetic Acid (*12*)—Official

12.034 APPARATUS

Insert thermometer reading to 0.1° into cork that fits $6 \times \frac{3}{4}''$ test tube and extend it far enough into tube so that bulb will be covered by 10 ml liquid. Place test tube in larger tube $(4 \times 1\frac{1}{4}'')$ contg glycerol and hold firmly in place with cork having groove cut in side to equalize pressure when heat is applied.

12.035 DETERMINATION

To remove traces of moisture, filter portion of sample to be examined thru dry paper in 110° oven. Let filtered sample cool until barely warm, and weigh 5 g sample and 5 g 99.5% HOAc into test tube. Insert cork holding thermometer and place test tube in glycerol bath. Heat and shake app. frequently until clear soln of the fat and HOAc is obtained. Let soln cool, with constant shaking, without removing from bath. Note temp. at which first sign of turbidity appears. Make similar test with same HOAc on sample of pure cacao butter.

As free fatty acids lower turbidity temp., correction must be made for acid value of sample. If concn of the HOAc reagent is such that turbidity temp. of pure cacao butter is ca 90°, one unit of acid value causes reduction of 1.4° in critical temp. of dissolution. If turbidity temp. is ca 100°, one unit of acid value causes reduction of 1.2°. For intermediate temp., reduction is proportional.

Det. acid value (mg KOH required to neutralize free fatty acids in 1 g sample) of both sample and pure cacao butter as in **26.042**, using 5 g fat. Multiply acid value by correction factor and add result to observed turbidity temp. Figure obtained is true critical temp. of dissolution. If this temp. is lower than that of pure cacao butter by >3° in case of fat from chocolate liquors or sweet chocolates, and by >6° in case of fat from milk chocolates, adulteration with coconut, palm kernel, corn, peanut, cottonseed oils, etc., or their stearins, is indicated.

12.036 Lecithin *(13)*—**First Action**

Weigh 5 g prepd sample, **12.001**, into 200 ml vol. flask, add ca 150 ml $CHCl_3$-absolute alcohol (1+1), and shake occasionally during day. At end of day dil. to vol. with same solvent, pour into 250 ml centrifuge bottle, stopper, and let stand overnight. Next day centrifuge stoppered bottle until clear (ca 15 min. at 1800 rpm). Pipet 100 ml clear liquid into 500 ml Kjeldahl flask. Place Kjeldahl flask on steam bath, remove solvent with current of air, and det. P_2O_5 as in **20.032** and **20.033**. $P_2O_5 \times 11.37$ = lecithin.

P_2O_5 may be detd by **6.064**, in which case conc. the 100 ml clear liquid in 250 ml beaker, wash into small crucible with solvent, evap., and proceed as in **6.063**. After digestion on steam bath, crucible must be heated cautiously on gauze until dry, and heating continued until frothing ceases and most of fat has smoked off before ashing in furnace. Ashing may be done in beaker in which ext. is evapd.

DAIRY INGREDIENT CONSTITUENTS

12.037 Milk Fat in Milk Chocolate—Official—*See* **26.027** and **12.029**

12.038 Milk Protein *(14)*—**Official**

Place 10 g milk chocolate in centrifuge bottle (250 ml or larger), and ext. twice with ca 100 ml ether by shaking until uniform, centrifuging, and decanting supernatant ether layer each time. Place in bottle perforated stopper carrying bent glass tube, and straight glass tube that extends into bottle ca ⅓ of way to bottom. Expel ether by applying suction to bent tube and drawing moderate current of air thru bottle while it is in moderately warm (not hot) place. When ether is expelled, pipet 100 ml H_2O into bottle. Stopper bottle, and shake vigorously 4 min. Pipet in 100 ml *1% $Na_2C_2O_4$ soln.* Stopper bottle, and shake vigorously 3 min. Let bottle stand ca 10 min. and again shake 1–2 min. Place sample in centrifuge and whirl ca 15 min. at high speed (ca 1800 rpm).

Remove bottle from centrifuge and decant supernatant into beaker. Pipet 100 ml into dry 250 ml beaker and add 1 ml HOAc while stirring gently. Let sample stand few min. so ppt can partly sep., and add with stirring 4 ml *10% tannic acid soln* (soln should not be more than 1 week old). Let ppt settle few min.; then filter on Coors No. 1A büchner with moderate suction. Filtrate should be clear. Use as filter S&S No. 589 white ribbon paper (or equiv.), overlaid with medium layer of paper pulp, prepd by shaking one 15 cm No. 1 Whatman paper, torn to bits, with H_2O. Using *wash soln* (add 1 ml HOAc and 2 ml 10% tannic acid soln to 100 ml 1% $Na_2C_2O_4$ soln), transfer all ppt to funnel with aid of policeman. Wash on filter 1 or 2 times. Loosen filter around edge with spatula. Carefully roll up and remove filter and ppt to Kjeldahl flask. Transfer to flask any particles of ppt clinging to funnel or spatula with small pieces of damp filter paper. Det. N as in **2.036**. $N \times 2 \times 6.38$ = total casein and albumin contained in 10 g taken for analysis. Casein and albumin $\times 1.07$ = total milk protein.

12.039 Lactose *(15)*—**Official**

(In absence of other reducing sugars)

Det. reducing sugars before inversion as in **29.039** in aliquot (usually 20 ml) of the Pb-free filtrate obtained in **12.040**. Det. reduced Cu as Cu_2O by volumetric thiosulfate method, **29.042**. Correct for Cu_2O due to sucrose as follows: Obtain approx. % lactose from following formula, using data obtained in **12.040**:

$$\text{Approx. lactose} = [P(1.1 + X/100) - S]/0.79.$$

From calcd polarimetric sucrose/lactose ratio and total Cu_2O obtained as above, det. quantity of Cu_2O to be subtracted from total Cu_2O found, using graph, Fig. 25. Convert corrected Cu_2O to

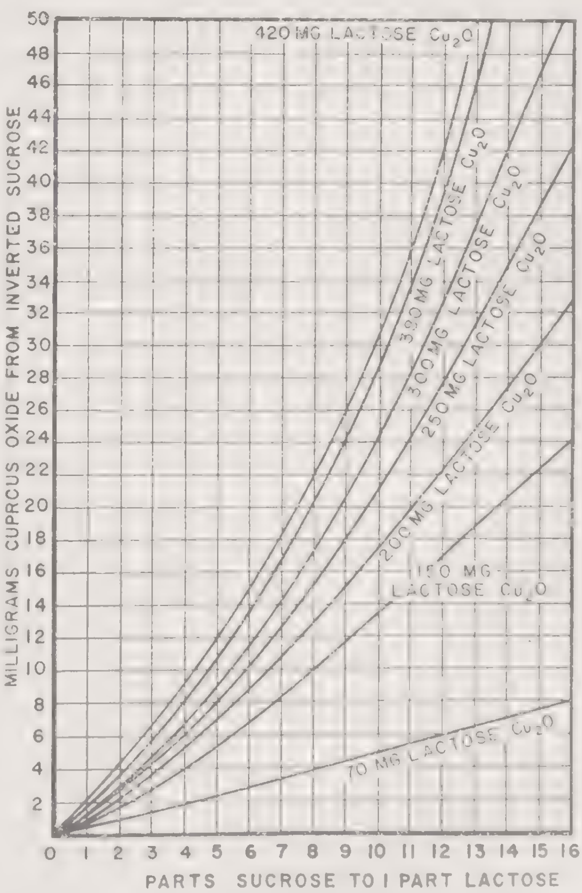

FIG. 25.—GRAPH USED IN CORRECTING CUPROUS OXIDE FOR EFFECT OF SUCROSE

g lactose (L), using table, **43.011.** Then obtain % lactose from following relationship:

% lactose $= L(110+X)/0.26C$, where X = value obtained in polarimetric sucrose detn and C = vol. soln (ml) used in above lactose detn.

SACCHARINE INGREDIENTS OTHER THAN LACTOSE

12.040 Sucrose *(15)*—**Official**

Transfer 26 g prepd sample, **12.001,** to 250 ml centrifuge bottle, add ca 100 ml petr. ether, shake 5 min., and centrifuge. Decant clear solvent carefully and repeat treatment with petr. ether. Place bottle contg defatted residue in warm place until petr. ether is expelled. Add 100 ml H_2O and shake until most of chocolate is detached from sides and bottom of bottle. Loosen stopper and carefully immerse bottle 15 min. in H_2O bath kept at 85–90°, shaking occasionally to remove all chocolate from sides of bottle. Remove from bath, cool, and add *basic $Pb(OAc)_2$ soln* (sp. gr. 1.25) to complete pptn (5 ml is usually enough). Add H_2O to make total of 110 ml added liquid. Mix thoroly, centrifuge, and decant supernatant thru small filter. Ppt excess Pb with powd. dry $K_2C_2O_4$ and filter. Dil. 10 or 20 ml filtrate with equal vol. H_2O, mix, and polarize in 200 mm tube at 20°. Obtain invert reading as in **29.026(b)**. Multiply both readings by 2 to obtain direct and invert polarizations "P" and "I." From data obtained calc. % sucrose (S) from following formulas:

$$S = \frac{(P - I)(110 + X)}{143.0 - t/2},$$

where

$$X = \frac{0.2244(P - 21d)}{1 - 0.00204(P - 21d)},$$

where

$$d = \frac{P - I}{143.0 - t/2}.$$

12.041 Dextrose *(16)*—**First Action**

Prep. clarified and deleaded sample soln as in **12.040** except to use only 10 g. Proceed as in **29.178** or **29.182**.

STARCH—FIRST ACTION

12.042 *Direct Acid Hydrolysis Method*

Weigh 4 g sample if unsweetened, or 10 g if sweetened, into small porcelain mortar; add 25 ml ether and grind. After coarser material settles, decant ether, together with fine suspended matter, on 11 cm paper of sufficiently fine texture to retain crude starch. Repeat treatment until no more coarse material remains. After ether has evapd from filter, transfer fat-free residue to mortar by means of jet of cold H_2O and rub to smooth paste, filtering on paper previously used. Repeat this process until all sugar is removed. (In case of sweetened products filtrate should measure at least 500 ml.) Det. crude starch in extd residue as in **22.043.**

12.043 *Diastase Method*

Remove fat and sugar from 4 g sample if unsweetened, or 10 g if sweetened, as in **12.042.** Carefully wash wet residue into beaker with 100 ml H_2O, heat to boiling over asbestos with constant stirring, and continue boiling and stirring 30 min. Replace H_2O lost by evapn and immerse beaker in H_2O bath kept at 55–60°. When liquid cools to bath temp, add 20 ml freshly prepd malt ext., **22.044,** and digest mixt. 2 hr with occasional stirring. Boil second time for 30 min., dil., cool, and digest as before with another 20 ml portion of the malt ext. Heat again to boiling, cool, and transfer to 250 ml vol. flask. Add 3 ml alumina cream, **29.021(b)**, dil. to mark, and filter thru dry paper. Residue on paper should show no signs of starch when examined microscopically. Continue as in **22.045,** beginning "Place 200 ml filtrate in flask, add 20 ml HCl (sp. gr. 1.125) . . ."

CHOCOLATE PRODUCTS

Alginates *(17)*—Official

12.044 REAGENT

Ferric hydroxide-sulfuric acid reagent.—Dissolve 10 g $FeCl_3.6H_2O$ in ca 100 ml H_2O in each of 2 centrifuge bottles, and ppt $Fe(OH)_3$ by adding excess NH_4OH (by odor). Wash ppt with ca 5 successive portions of H_2O, centrifuging and decanting until little odor of NH_3 remains. Break up centrifuged ppt each time before washing. Dry ppt on steam bath or in oven overnight, break up, and dry again. Mix with spatula or grind in mortar to obtain moderately fine powder. Keep in closed container.

Ferric hydroxide (moist) (code 1738, B&A, General Chemical Div., Allied Chemical & Dye Corp., New York, N. Y.) may be used instead of pptg $Fe(OH)_3$ as above. Transfer this product to centrifuge bottles, shake, centrifuge, decant, wash, and dry as above. Place 0.5 g dry powder in 50 ml g-s. graduated cylinder, add 50 ml H_2SO_4, shake vigorously, and let settle until clear (usually 4–7 days). Some ferric sulfate appears to stick to sides, but reagent is ready for use after 7 days. Prep. fresh after 3 weeks. Check as follows before use:

Dissolve small amount (1–5 mg) of commercial alginate in H_2O contg 5 drops 0.1N NaOH, add 4 vol. alcohol to ppt alginate, centrifuge, decant, and dry on steam bath until no odor of alcohol remains, using air current to remove last traces

of alcohol. Add 3 drops 0.1*N* NaOH, dissolve with aid of glass rod, and add 2 ml of the Fe-H_2SO_4 reagent. Purple color develops slowly, usually within 1 hr, depending on amount of algin present, but may take longer. If soln appears to be turning brown, add addnl 2 ml reagent, mix with glass rod, and let stand.

12.045 TEST

Weigh sample contg 10–20 mg alginate into 250 ml centrifuge bottle, add H_2O to total vol. of 40–50 ml, and dissolve by swirling. Adjust pH to 8–9 with *satd Na_3PO_4 soln*; usually 5 drops is enough. Add ca 0.5 g *pancreatin* and 3 drops HCHO, and shake vigorously 1 min. Let stand 2–16 hr.

Centrifuge at 1200 rpm 2–3 min., decant into 250 ml centrifuge bottle, and discard residue. Add 3–4 vols alcohol, shake, and let stand at least 1 hr, shaking several times. Centrifuge as before and discard liquid. Add 50 ml H_2O and 1 drop 10% NaOH to residue and shake vigorously until no more residue appears to dissolve. Add 3 g decolorizing C (Nuchar) and shake vigorously 1 hr, preferably on shaking machine. Do not centrifuge but pour directly into folded filter paper, collecting filtrate in 250 ml centrifuge bottle. If filtrate is not clear, pour back thru paper several times. If filtration is slow, let filter overnight. Since C retains some alginate, for recovery of very small amounts (ca 1 mg) re-ext. C by shaking with another 50 ml portion H_2O and 5 drops 0.1*N* NaOH, and add this filtrate to first extn.

To filtrate add 4 vols alcohol, shake, and let stand at least 1 hr, or overnight if convenient. Centrifuge and decant, saving residue. Residue contains alginates, gums, and gelatin. Dry residue on steam bath, using current of air, if desired, until no odor of alcohol can be detected. Cool, add 3 drops 0.1*N* NaOH, and dissolve residue, using glass rod, as completely as possible. Add 2 ml of the Fe-H_2SO_4 reagent, mixing with glass rod. If purple color develops very soon, enough reagent was added; if brown color appears, add addnl 2 ml reagent. Let stand overnight, since color develops slowly. Deep purple is positive test for alginates. If test is negative, repeat detn, using twice the size sample, increasing Nuchar to 4 g, and shaking 1.5 hr, for confirmation.

12.046 THEOBROMINE (*18*)—FIRST ACTION

(Not applicable to materials contg >ca 12% sweetening ingredients)

Ext. materials contg considerable fat, such as chocolate liquor or cacao nibs, with petr. ether (b.p. <65°) to remove fat. (This preliminary extn is unnecessary with samples of cocoas or cacao shell.)

Place 10 g sample or prepd sample in small porcelain dish. Add 2–3 g freshly *calcined MgO* and mix well with flat-end glass rod. Add ca 14 (9–20) ml H_2O, few ml at time, and triturate carefully and thoroly until every particle is damp. (Material should be compressible to firm cake.) Place dish contg damp mixt. on steam bath 30 min., mixing at intervals to prevent any part from becoming dry, during which time material should granulate.

After 30 min. remove dish and triturate mixt. well so that every particle is damp; then transfer to 250 ml flask. Add 150 ml *tetrachloroethane*, attach air condenser, and boil 30 min. Filter nearly boiling hot liquid into second ca 200 ml flask, preferably with ₮ joint. (Filtrate should be clear and almost colorless.)

Transfer residue and filter to first flask with 120 ml of the solvent and again reflux 20–30 min. Meanwhile, distill most of liquid in second flask from first extn thru air condenser. Filter hot liquid (second extn) into second flask and repeat process of refluxing and distn twice more, using 120 ml portions tetrachloroethane. Receive filtrates from all extns in flask 2, intermittently distg off portions as above. Distill liquid after last extn until reduced to 3–5 ml.

Cool flask and residue, and add 65 ml ether with rotation, mix well, stopper, and let stand at least 1 hr (until supernatant is clear). Collect ppt on tared filter paper, using several 5–7 ml portions ether to transfer and wash. Dry filter and ppt at 100°, and weigh. Add 0.004 g to wt found to compensate for theobromine dissolved in the ether. Calc. % theobromine in original material.

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(*4*) Ibid. **35,** 650(1952).
(*5*) Ibid. **14,** 526, 530(1931); **24,** 74, 720 (1941).
(*6*) Ibid. **8,** 705(1925); **9,** 469(1926).
(*7*) Ibid. **28,** 482(1945); **33,** 342(1950); **34,** 442(1951).
(*8*) Ibid. **13,** 43, 255(1930).
(*9*) Ibid. **11,** 45, 517(1928); **13,** 486(1930).
(*10*) Ibid. **11,** 517(1928); **13,** 45, 78, 486(1930).
(*11*) Ibid. **15,** 549(1932); **17,** 64, 375(1934).
(*12*) Ibid. **5,** 263(1921); **7,** 152(1923).
(*13*) Ibid. **32,** 167(1949); **35,** 656(1952); **36,** 263(1953).
(*14*) Ibid. **22,** 603(1939); **24,** 715(1941); **25,** 716(1942).
(*15*) Ibid. **16,** 564(1933); **17,** 65, 377(1934).
(*16*) Ind. Eng. Chem., Anal. Ed. **10,** 669(1938); J. Assoc. Offic. Agr. Chemists, **28,** 533(1945).
(*17*) J. Assoc. Offic. Agr. Chemists **40,** 47, 478 (1957); **42,** 370(1959).
(*18*) Analyst **46,** 35(1921).

13. Cereal Foods

WHEAT FLOUR (*1*)

13.001 Directions for Sampling—Official

Sample number of sacks equiv. to square root of number in lot, but not less than 10, *i.e.*, 10 from 100 or less, 15 from 225, 20 from 400 sacks, etc.

Select sacks to be sampled according to their exposure in ratio of 4 from most exposed, 3 from next less exposed, 2 from next, and 1 from least exposed portion of lot.

From each sack to be sampled, draw core from one corner of top diagonally to center of sack by means of cylindrical, pointed, polished metal trier, ½″ diam., with slit at least ⅓ of circumference. Draw second core from other top corner to ½ distance to center of sack.

Deliver the 2 cores at once to clean, dry, air-tight container that has stood open for few min. near lot of flour to be sampled, and seal immediately. Use sep. container for each sack sampled. Use one of following containers: (1) Pint fruit jar provided with rubber gasket; (2) rubber pouch that can be tied or sealed to exclude moisture or air; (3) tin can or box with moisture- and air-tight friction top.

Before opening sample for analysis, alternately invert and roll each container 25 times, or more if necessary, to secure homogeneous mixt. Avoid extreme temps and humidities when opening containers for analysis. Keep sample tightly sealed at all other times.

Total Solids (Moisture, Indirect Method)

(Also applicable to flour mixes contg $NaHCO_3$ as ingredient)

Vacuum Oven Method (2)—Official

13.002 APPARATUS

(**a**) *Metal dish.*—Diam. ca 55 mm, height ca 15 mm, with inverted slip-in cover fitting tightly on inside.

(**b**) *Air-tight desiccator.*—Reignited CaO is satisfactory drying agent.

(**c**) *Vacuum oven.*—Connect with pump capable of maintaining partial vac. in oven with pressure equiv. to 25 mm or less of Hg and provided with thermometer passing into oven in such way that bulb is near samples. Connect H_2SO_4 gas-drying bottle with oven to admit dry air when releasing vac.

13.003 DETERMINATION

Weigh accurately ca 2 g well-mixed sample in covered dish previously dried at 98–100°, cooled in desiccator, and weighed soon after attaining room temp. Loosen cover (do not remove) and heat at 98–100° to constant wt (ca 5 hr) in partial vac. having pressure equiv. to 25 mm or less of Hg. Admit dry air into oven to bring to atmospheric pressure. Immediately tighten cover on dish, transfer to desiccator, and weigh soon after it reaches room temp. Report flour residue as total solids and loss in wt as moisture (indirect method).

13.004 *Air-Oven Method (3)—Official*

(Results closely approximate those obtained by **13.003**)

In cooled and weighed dish (provided with cover) previously heated to $130 \pm 3°$, weigh accurately ca 2 g well-mixed sample. Uncover sample, and dry dish, cover, and contents 1 hr in oven provided with opening for ventilation and maintained at $130 \pm 3°$. (1 hr drying period begins when oven temp. is actually 130°.) Cover dish while still in oven, transfer to desiccator, and weigh soon after it reaches room temp. Report flour residue as total solids and loss in wt as moisture (indirect method).

13.005 Extract Soluble in Cold Water (*4*)—Official

Weigh 20 g flour into 500 ml erlenmeyer and add gradually 200 ml H_2O at ca 0°. Shake vigorously after ca 50 ml H_2O is added and continue shaking while adding remaining H_2O. Let mixt. stand 40 min. at 0°, shaking occasionally. Filter rapidly, returning first runnings to filter until filtrate is clear. Pipet 20 ml clear filtrate into weighed dish, evap. to dryness on steam bath, and dry in vac. oven at ca 100° for 30 min. periods to constant wt.

Ash (*5*)

13.006 *Direct Method—Official*

Weigh 3–5 g well-mixed sample into shallow, relatively broad ashing dish that has been ignited, cooled in desiccator, and weighed soon after reaching room temp. Ignite in muffle at ca 550° (dull red) until light gray ash results, or to constant wt. Cool in desiccator and weigh soon after it reaches room temp. Reignited CaO is satisfactory drying agent for desiccator.

Magnesium Acetate Method (6)—Official

13.007 REAGENT

Magnesium acetate soln.—Dissolve 4.054 g $Mg(OAc)_2.4H_2O$ in 50 ml H_2O and dil. to 1 L with alcohol.

13.008 DETERMINATION

From buret add 5 ml of the reagent to 3–5 g flour, bread, etc., or 10 ml to 1 g bran, wheat germ, etc. Let mixt. stand 1–2 min., evap. excess alcohol, and place in muffle maintained at 700°, closing door after flaming ceases. When incineration is complete, place dish in desiccator until cool; then weigh. Det. blank on soln and deduct blank from wt crude ash. Evap. blank cautiously.

Original Ash of Flour in Phosphated and Self-Rising Flour (7)

13.009 *Gustafson Method—Official*

To 20–25 g sample in metal centrifuge tube (cup 2″ diam., 6″ deep), add enough CCl_4 to fill tube to within 1″ of top (ca 250 ml). Centrifuge 5–7 min. at 1600 rpm and let centrifuge come to rest slowly. With large tablespoon, carefully skim off flour, which is in compact layer on surface of the CCl_4, recovering as much flour as possible in 1 spoonful. (With care, ca 90% of original flour may be recovered.) Let wet flour dry overnight and proceed as in **13.006**. (CCl_4 may be filtered, distd, and used again.)

13.010 Added Inorganic Material in Phosphated Flour (8)—Official

Transfer 20 g flour to dry 250 ml separator, add ca 200 ml CCl_4, shake well, and let stand until soln at bottom is nearly clear, usually ca 15 min. Draw off sediment with min. of soln, by turning stopcock quickly from side to side, into 100 ml CCl_4 in dry 125 ml separator. Again shake 250 ml separator and let stand, with occasional gentle swirling if necessary to dislodge sediment from sides, until lower portion of soln clears. Draw off sediment from 125 ml separator into prepd and weighed gooch, using suction. Draw off sediment from 250 ml separator into 125 ml separator as before, and let stand with occasional gentle swirling to dislodge sediment from sides of separator. After lower portion of liquid clears, draw off into gooch as before, taking care that no sediment remains on ledge in separator. Wash crucible and contents with 25 ml fresh CCl_4, continue aspirating 2 or 3 min., weigh at once, and report as % added phosphate. Ignite crucible at 700°, cool, and weigh as $Ca(PO_3)_2$. Wt $Ca(PO_3)_2 \times 1.27 \times 5 = \%$ $Ca(H_2PO_4)_2.H_2O$ in flour.

Iron (9)—Official

(Applicable to enriched, enriched self-rising, and phosphated flours)

13.011 REAGENTS

(a) *Orthophenanthroline soln.*—Dissolve 0.1 g *o*-phenanthroline in ca 80 ml H_2O at 80°, cool, and dil. to 100 ml.

(b) *Alpha, alpha-dipyridyl soln.*—Dissolve 0.1 g α,α-dipyridyl in H_2O and dil. to 100 ml.

(Keep reagents (a) and (b) in cool, dark place and they will remain stable several weeks.)

(c) *Hydroxylamine hydrochloride soln.*—Dissolve 10 g $NH_2OH.HCl$ in H_2O and dil. to 100 ml.

(d) *Magnesium nitrate soln.*—Dissolve 50 g $Mg(NO_3)_2.6H_2O$ in H_2O and dil. to 100 ml.

(e) *Acetate buffer soln.*—Dissolve 8.3 g anhyd. NaOAc (previously dried at 100°) in H_2O, add 12 ml HOAc, and dil. to 100 ml. (It may be necessary to redistill the HOAc and purify the NaOAc by recrystn from H_2O, depending on quantity of Fe present.)

(f) *2 Molar acetate buffer soln.*—Contg 272 g $NaOAc.3H_2O$/L.

(g) *Buffer soln, pH 3.5.*—Dil. 6.4 ml of the 2*M* acetate buffer soln, (f), and 93.6 ml 2*M* HOAc (120 g/L) to 1 L with H_2O.

13.012 PREPARATION OF STANDARD CURVE

(1) Dissolve 0.1 g analytical grade Fe wire in 20 ml HCl and 50 ml H_2O, and dil. to 1 L. Dil. 100 ml of this soln to 1 L. 1 ml = 0.01 mg Fe. Or—

(2) Dissolve 3.512 g $Fe(NH_4)_2(SO_4)_2.6H_2O$ in H_2O, add 2 drops HCl, and dil. to 500 ml. Dil. 10 ml of this soln to 1 L. 1 ml = 0.01 mg Fe.

Prep. 10 solns contg 2.0, 5.0, 10.0, 15.0, 20.0, 25.0, 30.0, 35.0, 40.0, and 45.0 ml, resp., of the final dild stock soln, plus 2.0 ml HCl, in 100 ml. Also prep. blank soln contg only the HCl. Using 10 ml of each of these solns proceed as in **13.013**, beginning "add 1 ml of the $NH_2OH.HCl$ . . . " Plot concn against scale reading.

13.013 DETERMINATION

(a) *By dry ashing.*—Ash 10.0 g flour in Pt, SiO_2, or porcelain dish (ca 60 mm diam., 35 ml capacity) as in **13.006**. (Porcelain evapg dishes of ca 25 ml capacity are satisfactory. Do not use flat-bottom dishes of diam. >60 mm.) Cool, and weigh if % ash is desired. Continue ashing until practically C-free. To diminish ashing time, or for samples that do not burn practically C-free, use one of following ash aids:

Moisten ash with 0.5–1.0 ml of the $Mg(NO_3)_2$ soln or with redistd HNO_3. Dry contents and carefully ignite in muffle to prevent spattering. (White ash with no C results in most cases.) Do not add these ash aids to self-rising flour (products

contg NaCl) in Pt dish because of vigorous action on dish. Cool, add 5 ml HCl, letting acid rinse upper portion of dish, evap. to dryness on steam bath, dissolve residue by adding 2.0 ml HCl, accurately measured, heat 5 min. on steam bath with watch glass on dish, rinse watch glass with H_2O, filter into 100 ml vol. flask, cool, and dil. to vol.

Pipet 10 ml aliquot into 25 ml vol. flask, and add 1 ml of the $NH_2OH.HCl$ soln; in few min. add 5 ml of the buffer soln, (**e**), and 1 ml of the *o*-phenanthroline or 2 ml of the dipyridyl soln, and dil. to vol. Read intensity of color in 2″ cell in neutral wedge photometer, using No. 51 filter (wavelength ca 510 mμ). (Other suitable instruments of equiv. precision may be used.) From reading det. Fe concn from equation of line representing std points or by reference to std curve for known Fe concn. Det. blank on reagents and make correction. Calc. quantity of Fe in flour as mg/lb. Rinse all flasks, beakers, funnels, etc., with H_2O before use, and filter all reagents to remove suspended matter.

(**b**) *By wet digestion.*—Transfer 10.00 g flour to 800 ml Kjeldahl flask, previously rinsed with dil. acid, then with H_2O; add 20 ml H_2O and mix; pipet 5 ml H_2SO_4 into flask and mix; add 25 ml HNO_3 and mix well. After few min. heat flask very gently at brief intervals (to avoid foaming out of flask) until heavy evolution of NO_2 fumes ceases. Continue to heat gently until material begins to char; then add few ml HNO_3 cautiously at intervals until SO_3 fumes evolve and colorless or very pale yellow liquid is obtained (60–65 ml HNO_3 in all in ca 2 hr). Cool, add 50 ml H_2O and 1 Pyrex glass bead, and heat to SO_3 fumes; cool, add 25 ml H_2O, and filter thru 11 cm paper into 100 ml vol. flask; rinse out flask, cool, and dil. to mark.

Pipet 10 ml into 25 ml vol. flask, add 1 ml of the $NH_2OH.HCl$ soln, rotate flask, and let stand few min. Add 9.5 ml of the 2*M* NaOAc soln, (**f**), and 1 ml of the *o*-phenanthroline soln, dil. to mark, and mix. Let stand at least 5 min. and read in 2″ cell in neutral wedge photometer or other instrument of similar precision.

With self-rising flour the 9.5 ml of the 2*M* NaOAc soln, (**f**), may be reduced to 8.0 ml. To det. exact amount of the buffer soln, (**f**), needed to adjust each digest to most desirable pH range, mix 10 ml aliquot of sample with measured amount of buffer soln, (**f**), dil. with H_2O to 25 ml, and det. pH either electrometrically or colorimetrically.

For colorimetric detn add 5 drops bromophenol blue indicator, **4.015**(**f**), to soln and compare color with that of equal vol. of the pH 3.5 buffer soln, (**g**), also treated with 5 drops of the indicator. Altho color develops from pH 2–9, avoid pH <3.0 and preferably work at pH 3.5–4.5. With cereal products the 9.5 ml of the buffer soln, (**f**), is satisfactory. With samples high in Fe, aliquot of 5 ml instead of 10 ml may be used with 4.8 ml of the buffer soln, (**f**). Conduct digestion so as to avoid contamination with Fe, and det. blank. After correction for blank, calc. as mg Fe/lb.

13.014 Calcium (*10*)—Official

(Applicable to enriched, enriched self-rising, and phosphated flours)

Ash 10 g flour or air-dried bread as in **13.006**, and proceed as in **13.013**(**a**) thru "rinse watch glass with H_2O . . . ", then filter into 400 ml beaker; or transfer 50 ml of the soln from Fe detn to 400 ml beaker. Dil. to ca 150 ml.

Add 8–10 drops bromocresol green indicator, **13.022**, and enough *20% NaOAc soln* to change pH to 4.8–5.0 (blue). Cover with watch glass and heat to boiling. Ppt Ca slowly by adding *3% oxalic acid soln*, 1 drop every 3–5 sec., until pH is 4.4–4.6 (optimum for Ca oxalate pptn) as indicated by distinct green shade. (Avoid excess of oxalic acid indicated by yellow tints, showing undesirable displacement of pH.) Boil 1–2 min. and let mixt. settle until clear or overnight. Filter supernatant thru quant. paper, gooch, or fritted glass filter (fine porosity), and wash beaker and ppt with ca 50 ml NH_4OH (1+50) in small portions, using wash bottle delivering very small stream. Break point of filter and wash filter or crucible with mixt. of 125 ml H_2O and 5 ml H_2SO_4 at 80–90°. Titr. at 70–90° with 0.05*N* $KMnO_4$ until slight pink is obtained, add filter paper, and continue titrn if necessary. Correct for blank and calc. Ca as mg/lb. 1 ml 0.05*N* $KMnO_4$ = 1 mg Ca.

Phosphorus (*11*)—Official

13.015 REAGENT

(**a**) *Magnesium nitrate soln.*—Dissolve 8 g MgO in HNO_3 (1+1), avoiding excess acid; add little MgO in excess, boil, filter from excess MgO, Fe_2O_3, etc., and dil. to 100 ml.

(**b**) Molybdate soln.—*See* **2.017**(**a**).

13.016 DETERMINATION

(**a**) Transfer 1.00 g sample to ca 140 ml porcelain casserole, add 3 ml of the $Mg(NO_3)_2$ soln, and mix well, using small glass rod. Clean rod with small piece of filter paper and place in casserole. Drive off most of moisture by drying in oven at 100° ca 2 hr, transfer to cold muffle, and ignite at 550° to white or gray ash (6–8 hr). Cool, cover with watch glass, take up with 10 ml HCl (1+4), and add 5 ml HCl. Rinse watch glass and evap.

to dryness on steam bath. Add 5 ml HCl and 50 ml H_2O, heat 15 min. on steam bath, filter into 100 ml vol. flask, cool, and dil. to vol. Pipet 50 ml into 300 ml erlenmeyer, neutralize to litmus paper with NH_4OH, make just faintly acid with HNO_3, dil. to 75–100 ml, add ca 15 g NH_4NO_3, and proceed as in **2.022**(a), beginning "Add . . . enough molybdate soln to insure complete pptn . . . " Or—

(b) Transfer 5.00 g sample to 35 ml porcelain evapg dish, mix well with 0.5 g Na_2CO_3, and ignite at 550° to gray ash. Cool, cover with watch glass, take up with 2 ml HCl (1+4), and add 5 ml HCl. Rinse watch glass, evap. to dryness, add 5 ml HCl and 10 ml H_2O, heat ca 10 min. on steam bath, filter into 100 ml vol. flask, cool, and dil. to vol. Pipet 10 ml aliquot into 300 ml erlenmeyer and proceed as in (a), beginning "neutralize to litmus paper with NH_4OH, . . . " Report results as % P.

13.017 Total Carbon Dioxide in Self-Rising Flour *(12)*—Official

(Not applicable to flours contg added $CaCO_3$)

Use 17 g flour, 15–20 glass beads (4–6 mm diam.), and 45 ml H_2SO_4 (1+5). Proceed as in **7.002–7.004,** as far as calcn, except to agitate flask vigorously 3 min. and let stand 10 min. to attain equilibrium.

Calc. as follows: Subtract vol. acid used from total buret reading and correct for temp. and pressure. Divide corrected reading by 100 to obtain % CO_2 (by wt). Correct apparent % CO_2 to compensate for varying atmospheric conditions by immediately assaying synthetic sample of known composition and like ingredients by same method in same app. Divide wt CO_2 recovered from synthetic sample by wt CO_2 contained in $NaHCO_3$ used and record quotient. Apparent % total CO_2 in official sample ÷ this quotient = corrected % total CO_2 in official sample.

13.018 Crude Fat or Ether Extract—Official

Proceed as in **22.033**; with fine flour, addn of equal wt clean, dry sand may be necessary.

13.019 Fat (Acid Hydrolysis Method) *(13)*—Official

Place 2 g sample in 50 ml beaker, add 2 ml alcohol, and stir to moisten all particles to prevent lumping on addn of acid. Add 10 ml HCl (25+11), mix well, set beaker in H_2O bath held at 70–80°, and stir at frequent intervals during 30–40 min. Add 10 ml alcohol and cool.

Transfer mixt. to Röhrig or Mojonnier fat-extn app. Rinse beaker into extn tube with 25 ml ether, added in 3 portions; stopper flask (with cork, Neoprene, or other synthetic rubber stopper not affected by solvents) and shake vigorously 1 min. Add 25 ml redistd petr. ether (b.p. <60°) and again shake vigorously 1 min. Let stand until upper liquid is practically clear, or centrifuge Mojonnier flask 20 min. at ca 600 rpm.

Draw off as much as possible of ether-fat soln thru filter consisting of pledget of cotton packed just firmly enough in stem of funnel to let ether pass freely into weighed 125 ml beaker-flask contg porcelain chips or broken glass. Before weighing beaker-flask, dry it and similar flask as counterpoise in oven at 100° and then let stand in air to constant wt.

Re-ext. liquid remaining in tube twice, each time with only 15 ml of each ether. Shake well on addn of each ether. Draw off clear ether solns thru filter into same flask as before and wash tip of spigot, funnel, and end of funnel stem with few ml of mixt. of the 2 ethers in equal vols free from suspended H_2O. Evap. ethers slowly on steam bath; then dry fat in oven at 100° to constant wt (ca 90 min.). Remove flask and counterpoise from oven, let stand in air to constant wt (ca 30 min.), and weigh. (Owing to size of flask and nature of material, there is less error by cooling in air than by cooling in desiccator.) Correct this wt by blank detn on reagents used. Report as % fat by acid hydrolysis.

13.020 Crude Fiber—Official—*See* 22.040

13.021 Fat Acidity *(14)*—Official—*See* 13.067

Hydrogen-Ion Concentration

Colorimetric Method—Official

13.022 PREPARATION OF SULFONPHTHALEIN INDICATORS *(15)*

	A	pH
Bromocresol green	14.3	3.8–5.4
Chlorophenol red	23.6	4.8–6.4
Bromothymol blue	16.0	6.0–7.6
Phenol red	28.2	6.8–8.4

A = ml 0.01N NaOH/0.1 g indicator required to form monosodium salt. Dil. to 250 ml for 0.04% reagent.

13.023 PREPARATION OF STOCK SOLUTIONS

Use recently boiled and cooled H_2O.

(a) *Acid potassium phthalate soln.*—0.2M. Dry to constant wt at 110–115°. Dissolve 40.836 g in H_2O and dil. to 1 L.

(b) *Monopotassium phosphate soln.*—0.2M. Dry KH_2PO_4 to constant wt at 110–115°. Dissolve 27.232 g in H_2O and dil. to 1 L. Soln should be distinctly red with Me red, and distinctly blue with bromophenol blue.

(**c**) *Boric acid-potassium chloride soln.*—0.2*M*. Dry H_3BO_3 to constant wt in desiccator over $CaCl_2$. Dry KCl 2 days in oven at 115–120°. Dissolve 12.405 g H_3BO_3 and 14.912 g KCl in H_2O, and dil. to 1 L.

(**d**) *Sodium hydroxide soln.*—0.2*M*. Should be as free as possible from carbonate. Dissolve 100 g NaOH in 100 ml H_2O, and let stand overnight till carbonate settles. Pipet clear soln from sediment and dil. to soln somewhat more concd than 1*N*. Stdze this soln with acid soln of known concn, or with $KHC_8H_4O_4$. From this approx. stdzn calc. quantity required for 0.2*M* soln. Make required diln with min. exposure and pour soln into Pyrex glass bottle. Carefully stdze soln as in **42.033**; 0.04084 g $KHC_8H_4O_4$ = 1 ml 0.2*M* NaOH. It is preferable to use factor with the soln rather than try to adjust to exactly 0.2*M*.

13.024 PREPARATION OF BUFFER SOLUTIONS

Max. range of std buffer solns usually needed in cereal work is pH 5.0–8.6. Prep. these from following stock solns, and in each case dil. to 200 ml.

Phthalate-NaOH Mixtures

pH	0.2*M* KH PHTHALATE (ML)	0.2*M* NaOH (ML)
5.0	50	23.65
5.2	50	29.75
5.4	50	35.25
5.6	50	39.70
5.8	50	43.10
6.0	50	45.40
6.2	50	47.00

KH_2PO_4-NaOH Mixtures

pH	0.2*M* KH_2PO_4 (ML)	0.2*M* NaOH (ML)
5.8	50	3.66
6.0	50	5.64
6.2	50	8.55
6.4	50	12.60
6.6	50	17.74
6.8	50	23.60
7.0	50	29.54
7.2	50	34.90
7.4	50	39.34
7.6	50	42.74
7.8	50	45.17
8.0	50	46.85

H_3BO_3-KCl-NaOH Mixtures

pH	0.2*M* H_3BO_3, KCL (ML)	0.2*M* NaOH (ML)
7.8	50	2.65
8.0	50	4.00
8.2	50	5.90
8.4	50	8.55
8.6	50	12.00

13.025 PREPARATION OF COLORIMETRIC STANDARDS

Place 20 ml of the buffer soln in ampuls ¾″ diam., or in test tubes of similar bore, and add 0.5 ml indicator soln. Do not keep unsealed ampuls or tubes more than few days because buffer solns may spoil.

13.026 DETERMINATION

To 10 g sample add 100 ml cool, recently boiled H_2O and digest 30 min. at 25°, shaking occasionally. Let mixt. stand quietly 15 min. and then decant supernatant thru folded, hardened, dry filter paper. Discard first 5 ml; then collect next three 20 ml portions in sep. tubes exactly like tubes holding colorimetric stds. Add 0.5 ml of proper indicator to one tube and compare resultant color with the prepd stds to det. pH.

Somewhat crude but helpful application of Walpole's principle to compensate for color and turbidity of sample can be made from block of wood. Bore parallel and in pairs, 6 deep holes, each large enough to hold one color std or sample tube. Place adjacent pairs as close together as possible without breaking thru intervening walls. Perpendicular to these holes and running thru each pair, bore smaller holes, thru which the test tubes may be viewed. Center pair of test tubes holds soln to be tested plus indicator and also H_2O blank. At each side place the stds colored with the indicator, and back each by sample of soln being tested. Place light on side of comparator contg the 2 controls and H_2O blank. If daylight is used, light from northern sky is best. If artificial light is used, it must not be too brilliant and should be passed thru daylight type of glass. For av. conditions, light intensity of 15–20 microamperes as registered thru photronic cell is adequate.

13.027 *Electrometric Method (16)—Official*

Weigh 10.0 g sample into clean, dry erlenmeyer and add 100 ml recently boiled H_2O at 25°. Shake contents of flask until particles are evenly suspended and mixt. is free of lumps. Digest 30 min., shaking frequently. Let stand 10 min. more, decant supernatant into the H-ion vessel, and immediately det. pH, using electrode and potentiometer stdzd by buffer solns of pH 4.01, **42.007**(**c**), and of pH 9.18, **42.007**(**e**), both at 25°.

Reducing and Non-Reducing Sugars (*17*)—Official

13.028 REAGENTS

(**a**) *Acetate buffer soln.*—Dil. 3 ml HOAc, 4.1 g anhyd. NaOAc, and 4.5 ml H_2SO_4 to 1 L with H_2O.

(**b**) *Sodium tungstate soln.*—12%. Dil. 12.0 g $Na_2WO_4.2H_2O$ to 100 ml with H_2O.

(**c**) *Alkaline ferricyanide soln.*—0.1*N*. 33.0 g pure dry $K_3Fe(CN)_6$ and 44.0 g Na_2CO_3/L.

(**d**) *Acetic acid-salt mixture.*—Dil. 200 ml

HOAc, 70 g KCl, and 40 g $ZnSO_4.7H_2O$ to 1 L with H_2O.

(e) *Soluble starch-potassium iodide soln.*—Add 2 g sol. starch to small quantity cold H_2O and pour slowly into boiling H_2O with constant stirring. Cool thoroly (or resulting mixt. will be dark colored), add 50 g KI, and dil. to 100 ml with H_2O. Add 1 drop NaOH soln (1+1). Use 1 ml.

(f) *Thiosulfate soln.*—0.1*N*. 24.82 g $Na_2S_2O_3.5H_2O$ and 3.8 g $Na_2B_4O_7.10H_2O$/L.

Make blank detn with each day's run of sugar detns to guard against changes in the $K_3Fe(CN)_6$ soln and correct for any reducing impurities in reagents as follows:

Combine 5 ml alcohol, 50.0 ml of the acid buffer soln, and 2 ml of the Na tungstate soln. To 5 ml of this mixt. (used in place of 5 ml flour ext.) add 10.0 ml of the $K_3Fe(CN)_6$ soln and proceed as for reducing sugars. (10.0 ml of the $Na_2S_2O_3$ soln should discharge the blue starch-I color.) If titrn falls within $10 \pm .05$ ml do not discard reagents but correct in subsequent sugar calcns by using $Na_2S_2O_3$ equiv. of 10 ml $K_3Fe(CN)_6$ soln (*i.e.*, ml $Na_2S_2O_3$ soln required in above titrn) instead of 10.0 as basis for subtraction.

13.029 DETERMINATION

(a) *Preparation of extract.*—Place 5.675 g flour in 100 or 125 ml erlenmeyer. Tip flask so that all flour is at one side; then wet flour with 5 ml alcohol. Tip flask so that wet flour is at upper side and add 50.0 ml of the acetate buffer soln, keeping

13.030 *0.1N Ferricyanide Maltose-Sucrose Conversion Table**

0.1*N* Ferricyanide Reduced	Maltose per 10 g Flour	Sucrose per 10 g Flour	0.1*N* Ferricyanide Reduced	Maltose per 10 g Flour	Sucrose per 10 g Flour
ml	*mg*	*mg*	*ml*	*mg*	*mg*
0.10	5	5	4.50	237	214
0.20	10	10	4.60	244	218
0.30	15	15	4.70	251	223
0.40	20	19	4.80	257	228
0.50	25	24	4.90	264	233
0.60	31	29	5.00	270	238
0.70	36	34	5.10	276	242
0.80	41	38	5.20	282	247
0.90	46	43	5.30	288	251
1.00	51	48	5.40	295	256
1.10	56	52	5.50	302	261
1.20	60	57	5.60	308	266
1.30	65	62	5.70	315	270
1.40	71	67	5.80	322	275
1.50	76	71	5.90	328	280
1.60	80	76	6.00	334	285
1.70	85	81	6.10	341	290
1.80	90	86	6.20	347	294
1.90	96	91	6.30	353	299
2.00	101	95	6.40	360	304
2.10	106	100	6.50	367	309
2.20	111	104	6.60	373	313
2.30	116	109	6.70	379	318
2.40	121	114	6.80	385	323
2.50	126	119	6.90	392	328
2.60	130	123	7.00	398	333
2.70	135	128	7.10	406	337
2.80	140	133	7.20	412	342
2.90	145	138	7.30	418	347
3.00	151	143	7.40	425	352
3.10	156	148	7.50	431	357
3.20	161	152	7.60	438	362
3.30	166	157	7.70	445	367
3.40	171	161	7.80	451	372
3.50	176	166	7.90	458	377
3.60	182	171	8.00	465	382
3.70	188	176	8.10	472	387
3.80	195	181	8.20	478	392
3.90	201	185	8.30	485	397
4.00	207	190	8.40	492	402
4.10	213	195	8.50	499	407
4.20	218	200	8.60	505	—
4.30	225	204	8.70	512	-
4.40	231	209	8.80	519	

* These values are arbitrarily given for 10 g flour altho detn is made on only 0.5 g flour.

soln from coming in contact with the flour until all is added to flask. Then shake flask to bring flour into suspension. Add immediately 2 ml of the Na tungstate soln and again mix thoroly. Filter at once (Whatman No. 4 or equiv.), discarding first 8–10 drops of filtrate.

(**b**) *Reducing sugars.*—Pipet 5 ml of the flour ext. into ca 75 ml test tube (Pyrex 1×8″). Add exactly 10 ml of the $K_3Fe(CN)_6$ soln to test tube, mix, and immerse test tube in vigorously boiling H_2O bath so that liquid in tube is 3–4 cm below surface of boiling H_2O.

After exactly 20 min. in boiling H_2O bath, cool tube and contents under running H_2O, and pour at once into 100 or 125 ml erlenmeyer. Rinse test tube with 25 ml of the HOAc-salt soln, add to erlenmeyer, and mix thoroly. Then add 1 ml of the starch-KI soln. Titr. with the $Na_2S_2O_3$ soln until blue color completely disappears (10 ml micro buret recommended). Subtract ml 0.1N $Na_2S_2O_3$ used in titrn from 10.00. In case of slight blank in the $K_3Fe(CN)_6$-$Na_2S_2O_3$ titrn, correct by subtracting from the $Na_2S_2O_3$ equiv. of the $K_3Fe(CN)_6$ soln. This difference represents definite quantity of reducing sugar/10 g flour, calcd as maltose from table, **13.030** (page 163).

(**c**) *Non-reducing sugars.*—Pipet 5 ml of the flour ext. into 8″ test tube and immerse in vigorously boiling H_2O bath. After boiling 15 min. cool test tube and contents under running H_2O and add exactly 10 ml of the $K_3Fe(CN)_6$ soln. Proceed as in (**b**). $K_3Fe(CN)_6$ reduced after hydrolysis $-K_3Fe(CN)_6$ reduced by maltose in flour = non-reducing sugars calcd as sucrose and detd from table, **13.030** (page 163).

13.031 Total Protein—Official

Det N as in **2.036**, and multiply % N by 5.7 to obtain % protein. Use factor 5.7 to convert N to protein in wheat used either for manufacturing purposes or for human food.

13.032 Water-Soluble Protein-Nitrogen Precipitable by 40 Per Cent Alcohol (*18*)—Official

Weigh 20 g sample (20-mesh or finer) into 250 ml centrifuge bottle. Pipet in 100 ml H_2O, shaking bottle to prevent lumping of sample. Add 100 ml more H_2O from pipet. Stopper bottle and shake 1 hr in shaking machine or by hand. (Preferably horizontal shaker with bottle lengthwise. If vertical wrist-type motion machine is used, shake by hand 5 min. after the 1 hr shaking.) Temp. of H_2O should be not >30°. Centrifuge at 1200 rpm ca 15 min. and filter into 500 ml suction flask thru pad of fine asbestos on büchner (ca 2″ diam.), using suction. Det. N in 50 ml filtrate as in **2.036** with glass bead in each flask, distg the NH_3 into 20 ml 0.1N acid. Digest 1 hr after clear. Correct for blank on reagents used in digestion.

Pipet 100 ml of above filtrate into 200 ml vol. flask, add 15 ml *NaCl soln* (28 g dild to 300 ml), fill nearly to mark with alcohol, mix well, cool to room temp., dil. to mark, mix, and let stand overnight. Pipet off supernatant and filter thru 18.5 cm fluted paper (S&S 588 or equiv.). Det. N in 100 ml filtrate as above, using glass bead to avoid bumping. Add the H_2SO_4, mix, and carefully boil off the alcohol before adding Na_2SO_4-HgO mixt. Rinse the Na_2SO_4-HgO mixt. down neck of flask. Digest 1 hr after clear. (Watch for foaming before clearing and keep contents out of neck of flask.) Distill into 20 ml 0.1N acid as before. Correct for blank on reagents used in digestion. Subtract this number of ml acid used from the number of ml acid used for H_2O-sol. N detn and convert to % H_2O-sol. N precipitable by 40% alcohol.

13.033 Lipoids (*19*)—Official

Add 15 ml alcohol, 70% by vol., to 5 g sample (20-mesh or finer) in 250 ml centrifuge bottle. Give bottle gentle rotary motion so as to moisten all particles, stopper firmly (to keep in place during heating), and set in H_2O bath kept at 75–80°. (Consider that temp. of bath may drop when bottles are introduced.) Heat 15 min., frequently mixing with same rotary motion. Immediately add 27 ml alcohol, stopper bottle, and *shake vigorously* 2 min. Cool, add 45 ml ether, and shake vigorously 5 min. (Sample should now be finely divided.) Centrifuge at ca 1000 rpm few min. and decant into 250 ml beaker contg some bits of broken porcelain or glass; rinse bottle neck with ether. Re-ext. sample with three 20 ml portions ether, shaking ca 2 min. each time, centrifuging, and decanting into beaker contg first ext. Break up sample each time with glass stirring rod, rinsing with ether on removal.

Evap. combined ether-alcohol exts just to dryness on steam bath. Drive off any remaining moisture on sides of beaker by placing in oven 5 min. at 100°. Dissolve dry ext. in ca 15 ml $CHCl_3$ and filter soln into previously dried and weighed 100 ml Pt dish thru asbestos mat 3–4 mm thick, covered with ca 10 mm layer of sand in Knorr type extn tube (20 mm diam.×11 cm long; stem 10 cm long). Wash sides of dish and tube with 10 ml and two 5 ml portions $CHCl_3$. Free with glass rod any solid ext. adhering to dish to be sure all lipoids dissolve. Finally wash tube and tip with 5 ml $CHCl_3$. Evap. $CHCl_3$ on steam bath and dry in oven at 100° to constant wt (ca 90 min.). Weigh. Report ext. as lipoids.

13.034 Lipoid Phosphorus *(19)*—**Official**

Wash sides of the Pt dish with 10 ml $CHCl_3$ to dissolve lipoids, **13.033**; likewise wash sides of dish with 10 ml *4% alc. KOH soln.* Evap. cautiously to dryness on steam bath and ash 1 hr at 500°. Cover dish with watch glass, add 15 ml HNO_3 (1+9) to make soln definitely acid, heat on steam bath ca 5 min., and filter into 300 ml erlenmeyer. Wash residue and filter with ca 25 ml hot H_2O. Make soln slightly alk. to litmus paper with NH_4OH from Mohr pipet and then slightly acid with HNO_3 (1+9). Keep vol. <ca 60 ml. Add 20 ml NH_4NO_3 soln, **20.029(b)**, and heat in H_2O bath to 45–50°. Add 20 ml freshly prepd and filtered molybdate soln, **2.020(a)**, and proceed as in **20.030,** line 9, beginning "and let flasks remain in bath 30 min. . . . " Det. blank and make correction.

13.035 Unsaponifiable Residue—Official
See **13.126**

Starch *(20)*—**First Action**

13.036 REAGENT

Calcium chloride soln.—Dissolve 2 parts $CaCl_2$ $.6H_2O$ in 1 part H_2O and adjust to density of 1.30 at 20° (soln contains ca 33% $CaCl_2$). Make faintly pink to phthln by adding 0.1*N* NaOH. (Anhyd. $CaCl_2$ may be used, but it is usually alk. and requires addn of acid to bring it to correct pH.)

13.037 DETERMINATION

Grind sample finely (100-mesh if possible) and weigh 2.0–2.5 g into 50 ml round-bottom centrifuge tube with lip. Wash with ether to remove fat, then with 10 ml ca 65% by wt alcohol (d_{20} 0.88), and stir thoroly with glass rod. Centrifuge (if no centrifuge is available wash samples on filter paper, using Pt cone and slight suction) and pour off soln. Repeat washing until 60 ml wash liquid has been used, stirring each time with same rod.

Stir residue with 10 ml H_2O and pour into 200–250 ml erlenmeyer. Complete transfer by washing with total of 60 ml of the $CaCl_2$ soln contg 2 ml 0.8% HOAc. Transfer rod to flask and bring mixt. to boiling quickly over wire gauze, stirring frequently. Boil briskly 15–17 min., taking precautions to prevent burning and foaming. Rub down particles on sides of flask with rod from time to time.

Cool soln quickly in running H_2O and pour into 100 ml vol. flask, rinsing thoroly with the $CaCl_2$ soln from wash bottle with medium jet. In dilg to mark, add 1 drop alcohol, if necessary to destroy froth.

After thoroly mixing sample pour ca 10 ml soln onto fluted filter (Whatman No. 42 or 44), wetting paper completely. Let filter run dry and discard filtrate. Resume filtration, using dry receiver, and collect 40–50 ml. As filtering aids, Celite with Pyrex glass filters or Hirsch-type funnel with asbestos and suction are recommended.

Polarize liquid in 10 cm tube, taking 2 sets of 10 readings each. (Averages of 2 sets should agree within 0.006°.)

$$\% \text{ starch} = \frac{100 \times A \times 100}{1 \times 200 \times S} = \frac{50 \times A}{S},$$

where A is observed angular rotation and S is wt sample. 200 is arbitrarily taken as specific rotation for all starches until better figure is detd for individual starches. If 200 mm tube and saccharimeter are used, 2 g sample weighed, and mixt. dild to 100 ml, °S×4.3225 = % starch.

13.038 Vitamins in Enriched Flours—
See **Chap. 39**

Chlorine in Fat of Flour

13.039 *Qualitative Test (Chlorine-Bleached Flours)—First Action*

Ext. 30 g flour with 50 ml petr. ether and let solvent evap. (Small quantity of oil remains.) Heat piece of Cu wire in colorless gas flame until it is black and no longer colors flame green. Dip hot end of wire into oil and again bring into flame. If Cl or Br has been used as bleaching agent, green or blue coloration is produced.

Quantitative Method (21)—Official

13.040 EXTRACTION OF FAT

Weigh 500 g flour into 2 L flask. Add 700 ml petr. ether and shake at 5 min. intervals 30 min. Filter thru büchner, pressing flour to obtain as much solvent as practicable. Transfer petr. ether ext. to large beaker and evap. on steam bath to ca 10 ml. Filter into container thru small funnel contg pledget of cotton packed firmly in stem. (Filtrate must be clear and free from flour.)

13.041 DETERMINATION

Heat ca 90 ml porcelain crucible contg 10 g *fusion mixt.* (138 g K_2CO_3, 106 g Na_2CO_3, and 75 g powd. KNO_3) 30 min. in 100° oven; dry in desiccator and weigh. Transfer the filtered 10 ml petr. ether ext. to crucible, using petr. ether for rinsing. Evap. petr. ether on steam bath and dry fat in 100° oven 30 min. Cool, and det. wt fat by difference. Add 5 g more of the fusion mixt. to crucible and spread evenly. Ignite to white ash in muffle at 525° (ca 1 hr) and cool.

Add 25 ml hot H_2O to mixt. and transfer with

small quantity hot H_2O to 200 ml tall beaker or beaker-flask. Add HNO_3 cautiously until soln is slightly acid to litmus paper. Add 25 ml more HNO_3. Add 5 ml 0.3*N* $AgNO_3$. Boil 5 min. in hood and cool to room temp. Filter thru 9 cm Whatman No. 1 paper, or similar Cl-free paper. Use 1% HNO_3 soln for rinsing. Digest as in **6.070**, beginning "Place paper and contents in Kjeldahl flask . . . " After digestion use 175 ml H_2O. Det. blank on reagents. Report Cl as mg/g fat.

Nitrite Nitrogen (*22*)—Official

13.042 REAGENTS

(**a**) *Sulfanilic acid soln.*—Dissolve 0.5 g sulfanilic acid in 150 ml HOAc (1+4), warming slightly if necessary.

(**b**) *Alpha-naphthylamine hydrochloride soln.*—Dissolve, by heating, 0.2 g of the salt in 150 ml HOAc (1+4).

(**c**) *Nitrite std soln.*—Dissolve 0.1097 g dry $AgNO_2$ in ca 20 ml hot H_2O, add 0.10 g NaCl, shake until AgCl flocculates, and dil. to 1 L. Draw off 10 ml of the clear soln and dil. to 1 L. 1 ml of this nitrite soln = 0.0001 mg N. Prep. just before use.

Prep. $AgNO_2$ as follows: To cold soln of ca 2 g $NaNO_2$ or KNO_2 in 50 ml H_2O, add soln of $AgNO_3$ as long as ppt forms. Decant liquid and thoroly wash ppt with cold H_2O. Crystallize from boiling H_2O and dry crystals in dark at room temp. (preferably in vac.).

13.043 DETERMINATION

Select series of 100 ml vol. flasks of uniform dimensions and color (125 ml erlenmeyers can be used). Place 2 g untreated (nitrite-free) flour in each flask. To flasks add 0, 5, 10, 15, 20, 25, 30, and 35 ml of the std $NaNO_2$ soln, resp., and dil. with H_2O to make 80 ml. Shake while adding the std soln and H_2O to moisten and disperse flour before mixt. becomes too dil.

Add 2 g flour sample to similar flask, and add 80 ml H_2O. Place flasks in H_2O bath at 40° and digest at least 15 min. Add 2 ml of the sulfanilic acid soln from Mohr pipet to each flask in succession, mix well, and add 2 ml of the α-naphthylamine hydrochloride soln. Continue digestion at 40° for 20 min. from time of addn to last flask. Shake samples occasionally during first 10 min. and let flour settle during last 10 min.

Remove from bath without disturbing settled flour. Compare unknown with series of stds and estimate closest match. Multiply ml std $NaNO_2$ soln in flask by 0.05 to obtain ppm N (*e.g.*, unknown may be between 30 and 35 ml, ca 32 ml; or $32 \times 0.05 = 1.6$ ppm N.

13.044 **Benzoyl Peroxide Bleach (Benzoic Acid)** (*23*)—**Official**

Place 50 g flour in (preferably) 500 ml g-s. erlenmeyer, and add 30–40 glass beads (ca 6 mm diam.), 0.1 g *powd. Fe*, and 100 ml ether. Let stand few min., shake with rotary motion, and add slowly (preferably dropwise) 2.5 ml HCl from Mohr pipet. Let stand ca 30 min., rinse down sides with small quantity of ether, and let stand overnight. Shake well with rotary motion, let flour settle few min., and decant thru 100 mm büchner, fitted with paper moistened with ether, into 500 ml suction flask. Add 50 ml ether, shake and let settle few min. Decant as before, repeat twice more, and after last addn, transfer whole contents to filter.

Transfer ether thru large funnel into 250 ml separator, add 20 ml *5% $NaHCO_3$ soln*, mix without too much vigorous shaking, and drain clear lower layer into 125 ml erlenmeyer. Repeat with one more 20 ml portion and two 10 ml portions of the $NaHCO_3$ soln. Add to this soln 0.3 g *Nuchar W*, shake, and filter (11 cm S&S 589 white ribbon, or equiv.) into 200 ml erlenmeyer. Wash flask and filter with 20–25 ml H_2O, using fine stream from wash bottle. Add 2.0 ml H_2SO_4 (1+1) dropwise to avoid foaming out of flask and swirl contents gently to reduce foaming. (Soln should be definitely acid to litmus paper.)

Transfer to 125 ml separator, rinse flask with 12 ml ether, and add to separator. Shake gently, frequently releasing pressure. (During first extn with ether, it is preferable to release pressure after each shake to avoid possible loss.) Repeat with 2 more 12 ml ether extns. Rinse flask each time with ether. After each extn drain aq. soln into same 200 ml erlenmeyer and transfer ether to ca 50 ml Pyrex test tube (25 mm diam. ×150 mm long). Add 2 ml 10% NaOH soln, hold top of tube firmly against palm of hand, and shake vigorously. Insert piece of *Cu wire* (1 mm diam. ×200 mm) into tube, and evap. ether very slowly on steam bath. Remove wire, place tubes in beaker of boiling H_2O, and evap. nearly to dryness. Add slowly up to 0.5 ml *30% H_2O_2*, followed by another 0.5 ml as soon as foam permits. (Min. frothing is desirable to permit better contact for nitration.) Break crust or film that forms before complete dryness by tapping tube against hands as evapn proceeds. Continue evapn to absolute dryness. (Introduction of gentle air blast into tube hastens evapn.)

Add, from Mohr pipet, 4 ml mixt. of H_2SO_4 and *fuming HNO_3* (1+1), taking care so that it washes down sides of tube, and heat 20 min. in gently boiling H_2O bath. Place slender glass rod in test tube, and occasionally rotate or rub rod against sides of tube to insure contact with

nitrating mixt. Immediately cool under tap to below room temp. and add 6 ml H_2O while keeping tube cool. Then *slowly* add 5 ml NH_4OH from Mohr pipet with continuous shaking under tap to keep soln cool. Add 10 ml more NH_4OH, keeping soln cool. Add 2 ml *6% $NH_2OH.HCl$ soln*, stir, and place in 65° H_2O bath 5–6 min., stirring occasionally. (Temp. of bath should be few degrees above, since cold tubes cause some decrease.) Cool to room temp. under tap, filter immediately thru folded paper into similar tube, and observe color of filtrate. Red or definite pink indicates presence of benzoic acid.

Transfer this soln (within 30 min.) to 2″ glass cell and read in neutral wedge photometer, using No. 51 filter, or other equally precise instrument set at 510 mμ. Prep. std curve by placing in test tubes 0.0, 0.4, 0.8, 1.0, and 1.2 mg *benzoic acid in acetone soln* (0.5 mg/ml). Add 2 ml 10% NaOH soln, shake to mix well, and proceed as in par. 3 beginning "place tubes in beaker of boiling H_2O, . . ." Report as ppm benzoic acid.

Bromates and Iodates in White and Whole Wheat Flour—Official

13.045 *Qualitative Test for Bromates and Iodates*

Cover bottom of white pan (ca 150 sq. in.) with reagent prepd by mixing equal vols HCl (1+7) and *1% KI soln.* Distribute evenly over liquid ca 4 g flour by sifting thru No. 60 sieve. Alternatively, sift flour over surface of dry pan and spray mixed reagent onto flour from glass atomizer until all particles are wetted. Black specks or purple spots not observed previous to addn of the reagent indicate presence of bromate or iodate.

13.046 *Qualitative Test for Iodates*

(**a**) *Applicable to 10 ppm or more.*—Distribute ca 1 g flour evenly over bottom of petri dish and completely cover with freshly prepd mixt. of 1 vol. *1% KSCN* to 4 vols HCl (1+32). Break up any lumps with stirring rod and observe with dish on white surface. Interpret results as in **13.045**.

(**b**) *Applicable to 1 ppm or more.*—Proceed as in **13.045** but use the acid-KSCN reagent (a).

Quantitative Method for Bromates (24)—Official

(Applicable in absence of iodates)

13.047 REAGENTS

(**a**) *Zinc sulfate soln.*—Dissolve 20 g $ZnSO_4.7H_2O$ in 800 ml H_2O and dil. to 1 L.

(**b**) *Sodium hydroxide std soln.*—0.4*N*. Dissolve 17 g NaOH in 1 L H_2O. Titr. soln against std acid and adjust to 0.4 ±0.01*N*.

(**c**) *Sodium hydroxide std soln.*—0.5*N*. Dissolve 21 g NaOH in 1 L H_2O. Titr. soln against std acid and adjust to 0.5 ±0.01*N*.

(**d**) *Dilute sulfuric acid.*—Approx. 4*N*. Add 112 ml H_2SO_4 to 800 ml H_2O. Cool, and dil. to 1 L.

(**e**) *Potassium iodide soln.*—Dissolve 25 g KI in 30 ml H_2O and dil. to 50 ml. Store in amber bottle in cool place. Discard soln showing yellow tinge of free I.

(**f**) *Ammonium molybdate soln.*—Dissolve 3 g $(NH_4)_6Mo_7O_{24}.4H_2O$ in 80 ml H_2O and dil. to 100 ml.

(**g**) *Potassium bromate stock soln.*—Dissolve 5.000 g $KBrO_3$ (dried 1 hr at 110°) in ca 800 ml H_2O and dil. to 1 L. 1 ml = 5 mg $KBrO_3$.

(**h**) *Potassium bromate std soln.*—Dil. 25 ml of the stock soln to 500 ml. 1 ml = 0.25 mg $KBrO_3$.

(**i**) *Potassium iodate stock soln.*—0.0898*N*. Dissolve 3.204 g KIO_3 (dried 1 hr at 110°) in ca 800 ml H_2O and dil. to 1 L.

(**j**) *Potassium iodate std soln.*—0.00359*N*. Dil. 10 ml of the stock soln to 250 ml. Prep. fresh daily.

(**k**) *Sodium thiosulfate stock soln.*—Dissolve 22.5 g $Na_2S_2O_3.5H_2O$ and 0.06 g anhyd. Na_2CO_3 in 800 ml H_2O, and dil. to 1 L. Dil. 10 ml to 250 ml. Transfer 5 ml dild soln to 200 ml erlenmeyer. Add 100 ml H_2O, 10 ml of the dil. H_2SO_4, and 1 ml of the KI soln. Add 5 ml freshly prepd starch soln, **2.093**(d), and titr. with 0.00359*N* KIO_3 from 10 ml buret graduated in 0.05 ml. Adjust stock $Na_2S_2O_3$ soln so that 10 to 250 diln is 0.00359*N*. Store stock soln in amber bottle in cool place.

(**l**) *Sodium thiosulfate std soln.*—0.00359*N*. Dil. 10 ml of the stock $Na_2S_2O_3$ soln to 250 ml. Prep. fresh daily and check titer at least monthly. 1 ml = 0.1 mg $KBrO_3$.

13.048 DETERMINATION

Transfer quantitatively 200 ml of the $ZnSO_4$ soln to 600 or 800 ml beaker and stir with speed-controlled, motor-driven glass stirrer. (Enough agitation to disperse flour is provided by vortex ca 1.5″ deep which does not extend to bottom of beaker.) Transfer 50 ±0.1 g sample to stirred $ZnSO_4$ soln in 2–5 g portions. Continue stirring ca 5 min., or until all dry flour on surface is uniformly dispersed in liquid. While stirring, add from pipet 50 ml of the 0.4*N* NaOH. Decrease speed of stirrer and stir ca 5 min. Filter or centrifuge, clarifying supernatant by filtration, if necessary. (24 cm Whatman No. 12 folded paper, or equiv. is satisfactory.)

Transfer 50 ml of this sample soln to 200 ml erlenmeyer. If smaller aliquot is taken, dil. to ca 50 ml with H_2O. Add 10 ml of the 4*N* H_2SO_4, 1 ml of the KI soln, 1 drop of the NH_4 molybdate soln, and 50 ml H_2O. While stirring, add 5–10 ml

$0.00359N$ $Na_2S_2O_3$ (an excess). Add 5 ml freshly prepd starch soln, **2.093(d)**, and titr. excess $Na_2S_2O_3$ with $0.00359N$ KIO_3. (Use 10 ml buret graduated in 0.05 ml for std solns. End point is best observed straight down.) As end point approaches, add KIO_3 soln slowly, 1 or 2 drops at time, swirling and viewing flask after placing it on white surface after each addn. Take first reddish or purple tinge as end point, and then add several more drops to confirm. Add addnl 1 ml of the $Na_2S_2O_3$ soln, and again titr. to addnl end point. Av. the 2 differences between amounts of $Na_2S_2O_3$ soln added and KIO_3 used in titrns. Ppm $KBrO_3$ $= 10 \times$(ml $0.00359N$ $Na_2S_2O_3$ − ml $0.00359N$ KIO_3). Correct results by recovery factor detd as below.

13.049 RECOVERY FACTOR

Dil. known vol. (x ml), >3 ml but <10 ml, of the std $KBrO_3$ soln to 250 ml. Using 50 ml aliquot, proceed as in second par. of detn. "Added bromate" in ppm $= 10 \times$(ml $0.00359N$ $Na_2S_2O_3$ − ml $0.00359N$ KIO_3).

Suspend 50 g portions non-bromated flour in two sep. 200 ml portions of the $ZnSO_4$ soln by stirring as above. To 1 (blank) suspension, add 10 ml H_2O; to other (recovery) suspension, add x ml std $KBrO_3$ soln and $(10 - x)$ ml H_2O. Continue as above except to add 40 ml $0.5N$ NaOH from pipet with continuous stirring. Use 5 ml std $Na_2S_2O_3$ for "blank" and 10 ml for "recovery." Deduct blank value, if any, from value of $KBrO_3$ found in "recovery" detn and multiply result by 10 to obtain ppm "recovered bromate."

Recovery factor = Added bromate/Recovered bromate.

13.050 Pigments in Flour (*25*) Official

Place 10 g flour in 125 ml g-s. flask and from pipet add 50 ml *H_2O-satd n-butyl alcohol*. Stopper flask tightly, shake well 1 min., and let stand 15 min. protected from sunlight. Reshake well and filter thru 12.5 cm folded paper (Eaton-Dikeman Co. No. 192, or equiv.), collecting filtrate in 50 ml erlenmeyer or suitable container. Fill 1 cm cell with the flour ext. and duplicate cell with corresponding solvent. Read absorbance at 435.8 mμ with spectrophotometer. From av. of 3 readings calc. pigment as carotene in ppm from std curve, or in absence of std carotene, from following formula:

$C = 5.0 \times \text{absorbance}/bK = 30.1 \times \text{absorbance}$, where C = pigment as carotene in ppm; b = cell thickness (cm); and $K = 0.16632$ (absorptivity (mg/L) for carotene at 435.8 mμ in H_2O-satd n-butyl alcohol).

Caution: Use strictly clean cells and filter thru paper the H_2O-satd n-butyl alcohol used as blank.

13.051 Diastatic Activity of Flour—Official

See **17.006**

13.052 Proteolytic Activity of Flour and Malted Wheat Flour—Official—*See* 17.011

Apparent Viscosity of Acidulated Flour-Water Suspension

By MacMichael Viscosimeter (26)—Official

13.053 ADJUSTMENT OF MACHINE

(**a**) Use No. 30 MacMichael viscosimeter wire.

(**b**) Have diam. of disk plunger $2.375 \pm 0.01''$.

(**c**) Adjust clearance between bottom of disk and inner surface of bottom of bowl to $0.25 \pm 0.005''$. Check clearance carefully with depth gauge reading in 0.001″.

(**d**) Use viscosimeter bowl with ca 7 cm diam. (depth of bowl will vary according to age of machine).

(**e**) Adjust regulating device to permit speed of exactly 12 rpm and check carefully and frequently with stop-watch, because as motor warms up machine tends to increase its speed.

(**f**) Adjust machine and keep it level, and when bob is placed see that it is riding freely and not touching sides of guide.

(**g**) Adjust dial so that when it comes to rest pointer is on zero mark.

13.054 PREPARATION OF LACTIC ACID

Add to concd lactic acid ca proportion of H_2O to give slightly stronger than $1N$ soln. Reflux this soln 3 hr, cool, and adjust to $1N$ by addg H_2O. Or proceed as follows: Use enough concd lactic acid to prep. soln ca $0.85N$ when stdzd with $0.1N$ NaOH. Transfer this soln to erlenmeyer fitted with air condenser to prevent undue evapn of H_2O, and heat 24 hr at 80° (soln will have increased in strength to ca $1.18N$). Adjust to exactly $1N$ with H_2O.

13.055 PREPARATION OF FLOUR-WATER SUSPENSION

In clean, dry, 500 ml erlenmeyer, place 20 g flour (15% moisture basis) and add 100 ml H_2O at 30°. Close with rubber stopper and shake vigorously 1 min. Place flask in constant temp. cabinet or H_2O bath 1 hr at 30°, shaking ca 10 times every 15 min. Remove flask from cabinet or H_2O bath, add 3 or 4 drops *capryl alcohol*, shake 10 times to remove any foam that may be present, and pour suspension into bowl of viscosimeter.

13.056 DETERMINATION

After pouring suspension into viscosimeter bowl, make sure bowl is flush on its supports. Start machine, but before placing bob or disk in

place, stir soln with bob 25 times to insure uniform suspension. Place wire of bob in holder and take reading after damping swing of dial by placing a finger on indicator pointer and then gradually touching swinging dial. Make second reading after adding 1 ml of the $1N$ lactic acid, and likewise third and following readings after adding 2 ml increments of the $1N$ lactic acid. Do not stop motor between readings. After or during addn of lactic acid, stir suspension 25 times by up-and-down motion of bob. Suspend bob by the wire and take reading. Det. max. apparent viscosity of the acidulated flour-H_2O suspension by plotting apparent viscosity readings against vol. acid added. Usually total of 7 ml of the $1N$ lactic acid is enough for max. reading, but 2 ml increments should be added continuously until apparent viscosity no longer increases.

Soybean Flour in Uncooked Cereal Products *(27)*

13.057 *Qualitative Test—First Action*

Place ca 0.5 g sample in small test tube contg strip of red litmus paper partly immersed in 5 ml *2% urea soln.* Mix, stopper tube, and heat 3 hr at 40°. If soybean flour is present in more than traces, litmus paper turns blue. (Bromothymol blue may also be used as indicator; it likewise turns blue if soybean flour is present.)

WHEAT, RYE, OATS, CORN, BUCKWHEAT, RICE, AND BARLEY AND THEIR PRODUCTS EXCEPT CEREAL ADJUNCTS *(28)*—OFFICIAL

13.058 PREPARATION OF SAMPLE

Grind sample to pass No. 20 sieve, or sieve having circular openings $\frac{1}{25}''$ (1 mm) diam., and mix thoroly.

13.059 **Moisture**—*See* **13.003**

13.060 **Ash**—*See* **13.006**

13.061 **Crude Fiber**—*See* **22.040**

13.062 **Iron in Degerminated, Bolted, Whole Corn Meal**—*See* **13.013**

13.063 **Crude Fat or Ether Extract**—*See* **22.033**

13.064 **Protein**—*See* **2.036**

(Protein = N × 6.25, except for wheat in which protein = N × 5.7)

Fat Acidity—Official

Method I. (14)

13.065 REAGENTS

(**a**) *Benzene-alcohol-phenolphthalein soln.*—To 1 L C_6H_6 add 1 L alcohol and 0.4 g phthln to form 0.02% soln.

(**b**) *Alcohol-phenolphthalein soln.*—To 1 L alcohol add 0.4 g phthln (0.04% soln).

(**c**) *Potassium hydroxide soln.*—$0.0178N$, CO_2-free. 1 ml = 1 mg KOH.

13.066 APPARATUS

(**a**) *Grain mill.*—Suitable for grinding small samples.

(**b**) *Fat extraction device.*—Soxhlet or other suitable type. (Double paper thimbles or Alundum RA-360 thimbles are suitable for extn.)

13.067 DETERMINATION

Obtain representative sample of ca 50 g of the grain (corn, 100 g) by hand quartering or by use of mechanical sampling device. Preferably grind sample so that at least 90% will pass No. 40 sieve (somewhat coarser grind will not materially affect results). If sample is too moist to grind readily, dry at temp. of ca 100° just long enough to remove excess moisture.

Ext. 10 ± 0.01 g ground sample with petr. ether ca 16 hr in the extractor. Start extn as soon as possible after grinding and under no circumstances allow ground sample to remain overnight. Completely evap. solvent from ext. on steam bath. Dissolve residue in extn flask with 50 ml of the benzene-alcohol-phthln soln. Titr. dissolved ext. with the KOH soln to distinct pink, or in case of yellow soln to orange-pink. If emulsion forms during titrn, dispel by adding second 50 ml portion of the benzene-alcohol-phthln soln. End point should match color of soln made by adding 2.5 ml *0.01% $KMnO_4$ soln* to 50 ml *$K_2Cr_2O_7$ soln* of proper strength to match color of original soln being titrd. (Add 0.5% $K_2Cr_2O_7$ soln dropwise to 50 ml H_2O until color matches. Then add 2.5 ml of the 0.01% $KMnO_4$ soln.)

Make blank titrn on 50 ml of the benzene-alcohol-phthln soln and subtract this value from titrn value of sample. If the addnl 50 ml portion of the benzene-alcohol-phthln soln was added, double blank titrn. Report fat acidity as mg KOH required to neutralize free fatty acids from 100 g grain (dry basis). Fat acidity = 10 × (titrn − blank).

13.068 *Method II. Rapid Method for Corn*

(Results may be obtained in <1 hr)

Prep. sample as in **13.067**. Weigh 20 ± 0.01 g into 100 ml g-s. flask or bottle. Add exactly 50 ml benzene, insert stopper, shake few sec. to sat. air in flask with benzene vapor, momentarily loosen stopper to release pressure, and replace stopper. Shake flask 30 min. in mechanical shaker, or periodically by hand 45 min. Tilt flask and let meal settle at an angle at least 3 min. Carefully

decant as much of liquid as possible into 15 cm folded paper inserted in 8 cm glass funnel, and cover funnel with cover glass to reduce evapn. Collect exactly 25 ml filtrate in 25 ml vol. flask. Then transfer this filtrate to 250 ml Florence flask. Refill vol. flask to 25 ml mark with the alcohol-phthln soln and transfer to flask contg benzene ext.

Using color std prepd as in **13.067,** titr. ext. with the std KOH soln to distinct pink in case of white corn, and to orange-pink for yellow corn. If emulsion forms during titrn, dispel by adding 25 ml each of benzene and of the alcohol-phthln soln. Det. blank titrn on mixt. of 25 ml benzene and 25 ml of the alcohol-phthln soln. If addnl benzene and alcohol were added, double blank titrn. Report fat acidity as mg KOH required to neutralize free fatty acids from 100 g corn (dry basis). Fat acidity = 10 × (titrn − blank), calcd on dry basis.

13.069 Vitamins in Enriched Grains—*See* Chap. 39

SOYBEAN FLOUR (*29*)—OFFICIAL

13.070 Moisture

Proceed as in **13.004,** except use 5 g sample and dry 2 hr.

13.071 Ash—*See* 22.010

13.072 Nitrogen—*See* 2.036

13.073 Crude Fiber—*See* 22.040

Oil or Petroleum Ether Extract—Official

13.074 REAGENT

Petroleum ether.—Initial boiling temp., 35–38°; dry-flask end point, 52–60°; at least 95% distg under 54°, and not >60% distg under 40°; sp. gr. at 60°F, 0.630–0.660; evapn residue not >0.002% by wt.

13.075 DETERMINATION

Weigh accurately duplicate samples of 2 g full-fat or 5 g low-fat soy flour, and wrap each portion in 150 mm filter paper (S&S No. 597 or equiv.); rewrap in second paper or papers so as to prevent escape of sample, leaving top of second paper open like thimble. Place piece of absorbent cotton in top of thimble to distribute the dropping ether. Place 25 ml of the petr. ether in 125 ml tared flask, and ext. sample 5 hr in Butt type or similar extractor. (Ether should drop on center of thimble at rate of at least 150 drops/min., and vol. solvent should be kept ca constant.) Evap. solvent until no trace remains, cool sample to room temp., and weigh. As last traces of ether are sometimes difficult to detect by odor, heat 1 hr, or longer, to constant wt.

BREAD

13.076 Preparation of Sample—Official

(When total solids of original loaf are not desired)

(**a**) *All types of bread not containing fruit* (*30*).—Cut loaf, or ½ loaf, of bread into slices 2–3 mm thick. Spread slices on paper and let dry in warm room until sufficiently crisp and brittle to grind well in mill. Grind entire sample to pass No. 20 sieve, mix well, and keep in air-tight container.

(**b**) *Raisin bread.*—Proceed as in (**a**), except comminute by passing twice thru food chopper instead of grinder.

13.077 Total Solids in Entire Loaf of Bread—Official

(**a**) *All types of bread not containing fruit* (*30*).—Accurately weigh loaf of bread immediately upon receipt (A), using scales sensitive to at least 0.2 g. If impossible to weigh accurately at this time, seal sample in air-tight container and accurately weigh as soon thereafter as is practicable (A). Preserve sample in such manner that no loss of bread solids can occur, whereby loss would be calcd as moisture.

Cut bread into slices 2–3 mm thick (½ loaf may be used). Spread slices on paper, let dry in warm room (15–20 hr), and when apparently dry, break into fragments. If bread is not entirely crisp and brittle, let it dry longer— until it is in equilibrium with moisture of air—so that no moisture changes may occur during grinding. Quantitatively transfer air-dried bread to scale pan and accurately weigh (B). Grind sample to pass No. 20 sieve, mix well, and keep in air-tight container. Det. % total solids (C) of ground sample as in **13.003** or **13.004.** Calc. total solids ($T.S.$) of bread from the formula:

$$T.S. = (100 \times B \times C/100)/A, \text{ or } B \times C/A,$$

where A = wt loaf (or ½ loaf) at time of receipt; B = wt air-dried sliced bread; and C = % total solids in prepd ground sample.

(**b**) *Raisin bread and bread containing raisins and fruit.*—Proceed as in (**a**), except comminute by passing twice thru food chopper instead of grinder and dry air-dried sample in uncovered dish ca 16 hr at 70° under pressure not >50 mm Hg.

13.078 Total Solids of Air-Dried Ground Sample (*30*)—Official

Use 2 g prepd sample, **13.076,** and proceed as in **13.003** or **13.004.**

13.079 Fat and Fat Number *(31)*— **Official**

Slice one loaf of bread, and let dry overnight, or until sufficiently dry to grind. Grind bread to ca size of openings on No. 20 sieve, mix, and transfer 50 g to 600 ml beaker. Add 100 ml H_2O and mix. Add 100 ml HCl, mix, cover, and heat on steam bath 1 hr, stirring well 6 or 7 times. Cool in cold H_2O bath (15° or less) and stir. Add 10 g Filter-Cel, or similar absorbent, stir, and mix completely. Prep. 90 mm büchner as follows:

Place two 9 cm S&S No. 590 filter papers (or equiv.) in funnel and apply suction. Mix 10 g Filter-Cel with 50 ml H_2O and rapidly pour mixt. into funnel. (This should make a smooth, even layer of Filter-Cel over paper, without cracks or openings.) Filter sample immediately. Rinse beaker several times with ice-cold H_2O. Just before filtration is complete, wash sides of büchner with ca 100 ml ice-cold H_2O (or until clear filtrate comes thru). Up to this point do not let pad suck dry. Continue with suction until Filter-Cel pad seems dry. Transfer this mass, without paper, from büchner to original beaker. Break up mass with rod, dry (overnight) on steam bath, and then heat in oven at 100° ca 30 min. to remove all moisture (material must be dry or fat results will be low). Break up any lumps.

Prep. large Knorr extn tube of ca 200 ml capacity (glass tubing 5 cm diam., 12 cm high from shoulder to top of tube). Pack tube with asbestos tamped tightly to form pad ca $\frac{3}{8}''$ thick. Insert stem of tube into 2 hole rubber stopper in filtering bell jar connected to suction thru 2-way stopcock. Place 500 ml erlenmeyer within bell jar so that stem of tube passes thru neck of flask. To cool beaker and contents, add 100 ml ether-petr. ether (1+1) and macerate 3–4 min. against sides of beaker with medium-size, stiff metal spatula. Decant into extn tube. Suck dry. Add 80 ml of the mixed ethers to beaker. Work as before 2 min. Transfer contents of beaker to extn tube, suck dry, and tamp with flat-end stirring rod until all ether is removed. To material in tube add 80 ml of the mixed ethers used just previously to rinse out beaker, mix thoroly with stirring rod few min., let stand 1 min., then suck dry, and tamp material as before. Make 2 addnl extns, turning suction on and off carefully to avoid loss of sample in erlenmeyer. Transfer to 1 L beaker. Evap. on steam bath, completely transfer fat with small quantities of petr. ether to tared 150 ml beaker, carefully evap. petr. ether on steam bath, dry at 100° to constant wt (ca 30 min.), cool, and weigh. Calc. % total fat on H_2O-free basis.

Weigh duplicate samples of 1 g (within ±.03 g) fat into 300 ml Florence flasks and add 4 ml glycerol-soda soln, **26.026(c)**. Heat flask carefully over asbestos gauze until bubbles start to appear; then hold flask ca 1″ over the heated gauze until cloudiness or turbidity disappears and mixt. is perfectly clear. After mixt. first becomes clear, 30–60 sec. addnl gentle heating insures complete saponification. Cool; add few pieces of previously ignited pumice stone, 138 ml CO_2-free H_2O, and 3 ml H_2SO_4 (1+4); and proceed as in **26.027,** using same app. Use 0.02*N* NaOH for titrn. Multiply ml 0.02*N* NaOH used by 1.1 and divide by wt fat used. Perform blank detn and make correction. Report number of ml 0.02*N* NaOH/g fat as "fat number."

Acetic and Propionic Acids *(32)*— **Official**

13.080 PREPARATION OF SAMPLE

(**a**) *Air-dried bread.*—Prep. sample as in **13.076–13.077.**

(**b**) *Fresh bread.*—For analysis of fresh product, which may be difficult to air-dry without spoilage or loss of volatile acids, pass sample thru meat grinder equipped with $\frac{1}{8}''$ hole plate, and divide finely by rubbing thru No. 8 sieve. Proceed with analysis promptly (24–48 hr) or preserve with $CHCl_3$ as follows:

To prepd bread in Mason jar filled to $\frac{3}{4}$ capacity, add washed $CHCl_3$ absorbed in ca 1 g cotton (ca 5 ml $CHCl_3$/pint container). Close jar tightly (self-sealing lids are recommended) and roll to mix contents thoroly. Store samples at ca 25° or refrigerate where higher temps occur.

13.081 REAGENTS—*See* **18.016**

13.082 APPARATUS

(**a**) *Distillation apparatus.*—Use volatile acid app., **18.015,** or gas-fired steam generator.

(**b**) *Chromatographic tubes.*—Approx. 15×250 mm or ca 15×450 mm, constricted at lower end to ca 4 mm i.d.

(**c**) *Test tubes.*—Approx. 16×150 mm, g-s.

(**d**) *Eyedropper pipet.*—Approx. 180 mm long.

13.083 DISTILLATION

Transfer 10 g of the air-dried bread or 15 g of the prepd fresh bread to 150 ml distg flask. Add 50 ml H_2O and 10 ml ca 1*N* H_2SO_4. Mix thoroly and add 10 ml *20% phosphotungstic acid soln.* Mix by swirling and add 40 g $MgSO_4.7H_2O$. Swirl again to partially dissolve salt. Mixt. should now be acid to *congo red paper;* if not, acidify with H_2SO_4 (1+1). Connect to condenser and steam generator, heat contents of distg flask to boiling, and distill 200 ml in 35–40 min. (Connect steam source just before heating bread solids suspension to prevent clogging of steam tube and to agitate bread solids.) Keep vol. in distg flask at ca 60–80 ml by means of small burner.

Transfer distillate to 400–600 ml beaker, add ca 10 ml *ca 0.01N formic acid,* make alk. to phthln with ca 1N NaOH, and evap. to ca 5 ml. Transfer to 25–30 ml g-s. test tube, rinsing beaker with 3 portions H_2O. If insol. material adheres, add few drops of ca 1N H_2SO_4 with 1 rinse. Make alk. to phthln and evap. just to dryness by inserting tube in steam bath or in boiling H_2O. (Air jet hastens evapn.) Det. acetic and propionic acids in evapd distillate by the sepn technic, **18.021(b)**(2) after prepg, testing, and stdzg column as in **13 084.**

13.084 CHROMATOGRAPHIC SEPARATION

(**a**) *Preparation of partition column.—See* **18.021(a)**. (Where amount of propionic acid approaches 20 mg in column and definite band is observed below propionic acid band, use the long chromatographic tube (450 mm) and ca 10 g silicic acid. Then take twice amounts of H_2O, indicator, and NH_4OH as used for 5 g silicic acid.)

(**b**) *Test of silicic acid for suitability and standardization of column.*—Prep. stock solns of formic, acetic, and propionic acids (reagent grade) by dilg 5 ml acid to 250 ml and stdze the acetic and propionic acids as in **18.021(b)**.

Prep. following dil. stock solns from the stock solns and boiled H_2O: Formic acid, 10 ml to 50 ml; acetic acid, 20 ml to 50 ml.

Prep. following trial mixts of formic acid and known amounts of acetic and propionic acids:

ACIDS AND WATER	MIXTURE A; STOCK SOLNS	MIXTURE B; STOCK SOLNS	MIXTURE C
	ml	*ml*	
Formic	10	10	1 ml dil. stock soln
Acetic	10	10	1 ml dil. stock soln
Propionic	10	30	1 ml stock soln
Water	20	None	None
Total vol.	50	50	3 ml
Test aliquot	1	1	3 ml

(Above mixts cover range of acetic and propionic acids usually present when 15 g fresh bread preserved with propionate is used as initial sample.)

Pipet indicated test aliquots from mixts A and B and entire mixt. C into bottom of g-s. test tubes (16×150 mm), neutralize with ca 1N NaOH, using phthln, and add 1 drop excess.

Proceed with evapn, sepn, and titrn as in **18.021(b)**(1) and (2). Calc. results for acetic and propionic acids to mg/100 g sample. Following factors are based on 15 g fresh bread or 10 g air-dried bread:

Fresh bread (mg/100 g):
Acetic acid $= 4.00 \times$ ml 0.01N $Ba(OH)_2$
Propionic acid $= 4.93 \times$ ml 0.01N $Ba(OH)_2$

Air-dried bread (mg/100 g):
Acetic acid $= 6.00 \times$ ml 0.01N $Ba(OH)_2$
Propionic acid $= 7.40 \times$ ml 0.01N $Ba(OH)_2$

(**c**) *Identification of acids.—See also* **18.021(c)**. With trial mixts A, B, and C and with most breads to which propionate has been added, only 3 definite bands appear on column and they elute in this order: propionic, acetic, and formic acids. Method provides for addn of enough formic acid to supply definite following band to insure that acetic acid is completely eluted. Amount of mobile solvent required to move each acid from top of column to point of emergence is function of concn of that acid. For amounts of acetic acid normally present in bread, differences in threshold vol. are not critical. To identify propionic acid by threshold vol., however, it is necessary to check threshold vol. for the concn indicated by titrn. Sepn of mixts A, B, and C supply enough data for amounts normally found. Threshold vols may be predicted for intervening concns by plotting concn against detd threshold vols.

Due to differences in propionic threshold vol., make change in mobile solvents from 1% butanol-$CHCl_3$ to 10% butanol-$CHCl_3$ at a constant vol. of 1% butanol-$CHCl_3$ rather than at propionic acid threshold, preferably when greater part of propionic acid has been eluted. Then acetic acid threshold vol. will not be affected by changes in propionic acid threshold vol.

Acids as sepd in butanol-$CHCl_3$ solns may be further identified by formation of mercurous acetate or mercurous propionate crystals (*33*).

13.085 Citric Acid (*34*)—**First Action**

To wt air-dried bread equiv. to 77.7 g moisture-free bread, in 500 ml vol. flask, add 400 ml *alc. H_2SO_4-phosphotungstic acid soln* (25 ml 1N H_2SO_4, 20 ml 20% phosphotungstic acid soln, and 55 ml H_2O, dild to 500 ml with alcohol). Shake 5 min., dil. to mark with the H_2SO_4-phosphotungstic acid soln, and let stand overnight. Readjust to mark with alcohol, shake 5 min., and filter with suction on paper in 12 cm büchner. Transfer 325 ml filtrate to centrifuge bottle, add 30 ml *$Pb(OAc)_2$ soln* (75 g of the salt + 1 ml HOAc dild to 250 ml with H_2O), shake 5 min., and centrifuge at ca 900 rpm 15 min. Decant supernatant (disregard turbidity), let drain, transfer residue with ca 150 ml H_2O to 250 ml vol. flask, and thoroly sat. with *H_2S*. Dil. to mark with H_2O, shake thoroly, and filter thru large folded paper. Proceed as in **20.049,** using 200 ml aliquot.

13.086 Ash (*35*)—**Official**

Use 3–5 prepd sample, **13.076**, and proceed as in **13.006** or **13.008**.

13.087 Chlorides in Ash—Official—*See* **13.116**

Iron—Official

13.088 PREPARATION OF SAMPLE

Slice bread, let air-dry until in equilibrium with air, and crush to ca 20-mesh size on wooden surface with wooden rolling pin. (Grinding may be done in mill if experiments show no increase in Fe due to grinding of particular material under examination. In general, grinding in mills increases Fe content.)

Proceed as in **13.013.**

13.089 Calcium—Official—*See* **13.014**

Vitamins in Enriched Bread—Official

13.090 PREPARATION OF SAMPLE

Det. fresh wt of entire sample taken for drying, usually 6 loaves of 1 lb size or alternate slices from 6 loaves of 1.5 lb size. Slice unsliced bread into slices ca 1 oz each. Spread slices on coarse screens, elevated to provide good air circulation, and let air-dry until crisp enough for efficient grinding. (If riboflavin is also to be detd, drying should be done in absence of light.) Weigh air-dried bread and grind entire amount to pass No. 20 sieve. Mix well, and store in air-tight glass jars at ca 10°. Det. air-dry wt:fresh wt ratio for subsequent use in calcn of results to fresh basis.

Proceed as in **39.029–39.032, 39.033–39.036,** and **39.037–39.038.**

13.091 Protein—Official

Det. N as in **2.036,** using 2 g prepd air-dried ground sample, **13.076.** Multiply % N by factor 5.7 to obtain % protein.

13.092 Fat (Acid Hydrolysis Method)—Official—*See* **13.019**

13.093 Sterols (As Cholesterol)—Official

Weigh 5 g air-dried, ground sample and proceed as in **13.128.**

13.094 Crude Fiber—Official

(For bread and other baked products not contg fruit)

Proceed as in **22.040.**

13.095 Sugars—Official—*See* **22.041** and **22.042**

Lactose (*36*)**—First Action**

13.096 APPARATUS

(a) *Rack.*—Metal rack so constructed as to prevent agitation of tubes while in boiling H_2O bath.

(b) *Titration stirrer.*—For stirring soln during titrn: rod made from glass tubing, sealed and flared at lower end to form button-like foot, is convenient. Make side arm consisting of several layers adhesive tape attached near enough to top of tube to prevent breaking bottom of titrn tube.

13.097 REAGENTS

(a) *Yeast suspension.*—Wash 25 g fresh commercial bakers' yeast with five 100 ml portions of H_2O or until last washings are clear. Centrifuge and decant after each wash. Suspend in 100 ml H_2O and store 24 hr at 0–4° before use. Prepns as old as 1 week are satisfactory.

(b) *Yeast nutrient soln.*—Dissolve 1.7 g Bacto peptone (Difco Laboratories), 0.50 g K_2HPO_4, and 0.33 g $MgSO_4.7H_2O$ in H_2O and dil. to 100 ml with H_2O.

(c) *Protein precipitant.*—Dissolve 50 g Na tungstate and 6 g Na_2HPO_4 in 200 ml H_2O. Slowly add 220 ml 2*N* HCl, mix, and dil. to 500 ml with H_2O.

(d) *Somogyi's reagent.*—Dissolve 12 g Rochelle salt, 20 g Na_2CO_3, and 25 g $NaHCO_3$ in ca 500 ml H_2O and pour into soln, with stirring, 6.5 g $CuSO_4.5H_2O$ dissolved in ca 100 ml H_2O; add soln of 10 g KI, 0.80 g KIO_3, and 18 g $K_2C_2O_4.H_2O$, and dil. to 1 L. (Only KIO_3 need be weighed accurately.) Let stand few days; then filter off any small amount of ppt.

(e) *Sodium thiosulfate soln.*—0.005*N*. Prep. daily by dilg freshly stdzd 0.1*N* soln.

13.098 DETERMINATION

Weigh 10 g air-dried bread, add 5 g Filter-Cel, and mix well. Transfer mixt. to extn thimble (ca 30×77 mm), cover with cotton pad, place in Soxhlet extractor, add 150 ml alcohol-H_2O mixt. (126 ml alcohol+61 ml H_2O), and ext. overnight on hot plate set at medium heat. Transfer ext. to 250 ml beaker (previously marked at 40 ml), evap. on steam bath with aid of weak air blast to ca 40 ml, and transfer to 100 ml vol. flask, rinsing well. Cool, dil. to vol., and mix well. Pipet 10 ml aliquot into 50 ml erlenmeyer; add 6 ml yeast suspension and 5 ml yeast nutrient soln. Prep. blank test, using 10 ml H_2O in place of bread ext. Stopper flask with 1-hole rubber stopper fitted with piece of 6 mm (not smaller) glass tubing ca 10 cm long. Shake at moderate rate 2.5 hr in constant temp. H_2O bath at 30°.

Transfer to 50 ml centrifuge tube and centrifuge ca 10 min. at ca 1000 rpm. Decant supernatant into 50 ml vol. flask. Rinse erlenmeyer with 10 ml H_2O, decanting onto residue in centrifuge tube. Mix residue and H_2O with glass rod. Centrifuge, and combine washing with previous supernatant, in 50 ml vol. flask. Repeat washing, using 10 ml H_2O. Add, with shaking 2.5 ml protein precipitant. Dil. to vol., mix well, and filter, discarding first few ml filtrate. (This is convenient stopping point; stopper flask for continuation next day.)

Pipet 5.0 ml clear filtrate into Pyrex test tube (22×175 mm) and neutralize to phenol red end point with 0.5*N* NaOH. Add 5.0 ml Somogyi's reagent, mix by rotary motion, and add 2 drops benzene. Cap tube with glass bulb and place in the metal rack. Immerse rack contg tubes in vigorously boiling H_2O bath for exactly 15 min. Cool, avoiding agitation, to ca 35° in H_2O bath. Add 2.5 ml 2*N* H_2SO_4, shake with rotary motion, let stand ca 1 or 2 min., and titr. the excess I with 0.005*N* $Na_2S_2O_3$, adding 6 drops of 1% starch indicator near end of titrn. (Titrns should be finished in 30 min.) From difference between titrn value of blank (H_2O, yeast suspension, and yeast nutrient) and that of sample, det. quantity of lactose present from std curve. This value in mg lactose represents amount in 100 mg air-dry bread or % lactose.

Prep. std curve, using from 0 to 4.5 mg pure *lactose hydrate* (0, 0.2, 0.5, 1.0, 2.0, 3.0, 4.0, and 4.5 mg from portions of soln contg 1.0 mg per ml) in enough H_2O to make 5 ml. Add 5.0 ml Somogyi's reagent, mix by rotary motion, and add 2 drops benzene. Proceed as above from "Cap tube . . . " Plot difference between titrn value for 0 mg lactose and that for each lactose soln against corresponding lactose concn in mg.

13.099 Hydrogen-Ion Concentration—Official—*See* 13.026 or 13.027

BAKED PRODUCTS OTHER THAN BREAD (NOT CONTAINING FRUIT) (*37*)

13.100 Solids—Official—*See* 13.077

13.101 Ash—Official—*See* 13.006

13.102 Protein—Official—*See* 13.091

13.103 Fat—Official—*See* 13.019

13.104 Sterols (As Cholesterol)—First Action

Weigh 5 g air-dried, ground sample and proceed as in **13.128**.

13.105 Crude Fiber—Official—*See* 22.040

13.106 Sugars—First Action—*See* 22.041 and 22.042

13.107 Hydrogen-Ion Concentration—Official—*See* 13.026 or 13.027

13.108 Acetic and Propionic Acids in Cake (*32*)—Official

Proceed as in **13.083**, using 15 g sample prepd as for fresh bread or fresh bread preserved with $CHCl_3$.

FIG BARS AND RAISIN-FILLED CRACKERS (*38*)

13.109 Moisture—Official

Place 25–30 g prepd sand and short stirring rod in dish ca 55 mm diam. and 40 mm deep, fitted with cover. Dry thoroly, cover dish, cool in desiccator, and weigh immediately. Remove cover, place 3–5 g prepd sample, **13.076(b)**, in dish, and weigh accurately. Remove dish contg the sand, stirring rod, and weighed sample from balance. Add 5–10 ml H_2O and mix with the sand. Heat carefully on H_2O bath, stirring at 2–3 min. intervals, until excess H_2O is removed and contents of dish are consistency of heavy paste. Place uncovered dish in vac. oven and dry ca 16 hr at 70° under pressure not >50 mm Hg. After drying, cover dish, transfer to desiccator, cool to room temp., and weigh immediately.

Notes: Quartz sand that passes No. 40 sieve but is retained on No. 60 sieve, has been digested with HCl, washed free of acid, and ignited, is recommended. Al dishes with fit-over covers are most convenient. Dish can be set in cover during heating on H_2O bath and during oven-drying period. After drying, cover can be easily and quickly refitted on dish as it is transferred to desiccator.

13.110 Fat—Official

Weigh accurately ca 2 g well-mixed sample, prepd by grinding twice thru food chopper, and transfer to Mojonnier tube. Add 2 ml alcohol, warm to 60–70°, and shake gently until sample is thoroly disintegrated and mixed with the alcohol. Add 10 ml HCl (25+11). Place tube in H_2O bath held at 70–80° and shake frequently until sample is thoroly digested (40–80 min.).

If weighed sample cannot be transferred to tube before it is digested, or if Röhrig tube is used, digest in 50 ml beaker. Transfer digested mixt. to tube as completely as possible by draining from lip of beaker down small stirring rod. Rinse beaker thoroly with 10 ml alcohol, transfer to extn tube, mix thoroly, and cool. Rinse beaker with portions of first 25 ml ether added for first extn. Repeat rinsing with portions of petr. ether (b.p. <60°) as it is added for first extn. Rinse thoroly so that all fat is transferred to extn tube. (After digestion, all particles should be completely disintegrated, except hard seeds (in fig fillers) and strong fibers. Very small quantity of fat may be retained by such particles after digestion, but in analysis of biscuits and crackers this loss will be within experimental error.)

When digesting in extn tube, add 10 ml alcohol to digested charge and cool. (Level of liquids should be in neck of Mojonnier tube just below

pour-off level or just below draw-off spigot of Röhrig tube.) Add 25 ml ether, stopper flask with cork or Neoprene or other synthetic rubber stopper not affected by solvents, and shake thoroly ca 1 min. Release pressure carefully so that none of solvent contg fat is lost. Wash adhering solvent and fat from stopper into extn tube with few ml petr. ether. (Wash bottle producing fine jet is convenient.) Let mixt. stand few min.; then add 25 ml petr. ether (b.p. $<60°$), stopper tube tightly, and again shake thoroly ca 1 min. Release pressure carefully, remove stopper, and again wash adhering solvent and fat into tube with few ml of the petr. ether. Let mixt. stand until ether layer is clear (10–20 min.), or centrifuge Mojonnier flask 20 min. at ca 600 rpm.

Pour off as much as possible of clear ether-fat soln thru small, fast filter by tilting Mojonnier tube gradually, or draw off thru spigot of Röhrig tube. (Plug of ether-extd cotton packed just firmly enough in stem of a funnel to let ether pass freely makes excellent filter for these extns.) Catch ether-fat solns from extns in clean 250 ml beaker or flask. Re-ext. digested sample remaining in tube 3 times more as for first extn. (Vol. of ether may be reduced to 15 or 20 ml for last 3 extns.) Wash mouth of Mojonnier tube or spigot of Röhrig tube each time after draining ether-fat soln, and filter this ether thru funnel into receptacle.

Evap. combined ethers from extns by fanning or suction. After ethers are practically off, heat ca 10 min. on hot H_2O or steam bath to drive off most of alcohol and H_2O carried over with ethers. Transfer beaker to 100° oven, dry 1 hr, remove, and let cool. Redissolve dried fat in 15–20 ml mixt. of equal parts of ether and petr. ether, and filter thru small fat-free paper into beaker or flask previously dried at 100°, cooled in desiccator, and weighed.

Wash all traces of fat from first receptacle, filter paper, and funnel into the tared beaker or flask with jet of petr. ether from wash bottle. Evap. ethers from tared receptacle by fanning or suction and dry purified fat to constant wt in 100° oven (1–1.5 hr). Cool in desiccator and weigh as soon as room temp. is attained. Make blank detns on reagents.

NOTES: Good quality rubber stoppers thoroly cleaned with alcohol are satisfactory for stoppering extn tubes. Remove stoppers from tubes after each shaking period and do not allow to remain in contact with solvents longer than necessary. Solvent may have some action on rubber. Very fine grain cork stoppers washed with alcohol and ether are also satisfactory for stoppering extn tubes, provided leakage of solvents can be prevented during shaking. Ground glass stopper in Röhrig tube is most satisfactory.

If trouble is experienced in releasing pressure after shaking extn tube contg ethers, cool tube slightly by holding it under stream of cold H_2O before removing stopper.

Al beakers are very satisfactory for weighing purified fat: they are light in wt and cool to room temp. rapidly.

MACARONI, EGG NOODLES, AND SIMILAR PRODUCTS

13.111 Collection and Preparation of Sample (*39*)—Official

Select from lot to be analyzed enough strips or pieces to assure representative sample, break these into small fragments with hands or in mill, and mix well. Grind 300–500 g in mill until all material passes thru No. 20 sieve. Keep ground sample in sealed container to prevent moisture changes.

Total Solids and Moisture

13.112 *Vacuum Oven Method (40)—Official*

Use 2 g prepd sample, **13.111**, and proceed as in **13.003.**

13.113 *Air Oven Method—Official*

Use 2 g prepd sample, **13.111**, and proceed as in **13.004.**

13.114 Ash—Official

Use 3–5 g prepd sample, **13.111**, and proceed as in **13.006.**

13.115 Original Ash in Macaroni Products Containing Added Salt But Not Containing Added Eggs (*41*)—First Action

Proceed as in **13.006.** Dissolve ash in 25 ml HNO_3 (1+3), transfer to 150 ml beaker, dil. to 75 ml with H_2O, and boil 15 min., maintaining original vol. (necessary to convert all phosphate to ortho form). Det. P_2O_5 as in **2.022.** $P_2O_5 \times 2$ = NaCl-free ash.

13.116 Chlorides in Ash as Sodium Chloride (*42*)—Official

Dissolve ash obtained in **13.114** in HNO_3 (1+9), filter, wash paper with hot H_2O, and det. Cl in combined filtrate and washings as in **6.066** or **6.068.** Calc. Cl to its equiv. of NaCl. (This NaCl value deducted from total ash gives only approx. NaCl-free ash.)

13.117 Iron—Official—*See* 13.013

13.118 Vitamins in Enriched Macaroni and Noodle Products—*See* Chap. 39

13.119 Fat (Acid Hydrolysis Method) (*43*)—Official

Place 2 g sample in Röhrig or Mojonnier extn tube, add 2 ml alcohol to prevent lumping on addn of acid, and shake to moisten all particles. Add 10 ml HCl (25+11), mix well, set tube in H_2O bath held at 70–80°, and shake at frequent intervals during 30–40 min. Fill to within 1–2 ml of mark with alcohol and cool. Add 25 ml ether and shake mixt. well. Then add 25 ml petr. ether (b.p <60°) and mix well. Let stand until upper liquid is practically clear and proceed as in **13.019,** beginning "Draw off as much as possible . . ."

13.120 Crude Fiber—Official—*See* 22.040

13.121 Protein (*44*)—Official

Det. N as in **2.036,** using 1 g prepd sample, **13.111.** % protein = % N × 5.7.

13.122 Water-Soluble Protein-Nitrogen Precipitable by 40 Per Cent Alcohol—Official—*See* 13.032

13.123 Hydrogen-Ion Concentration—Official—*See* 13.026 or 13.027

13.124 Lipoid and Lipoid Phosphorus—Official

Proceed as in **13.033** and **13.034.**

Unsaponifiable Residue (*45*)—Official

13.125 REAGENTS AND APPARATUS

See **16.013** and **16.014.** (The concd KOH soln is not needed.)

13.126 DETERMINATION

Weigh 10 g sample, ground to pass No. 20 sieve, into 500 ml erlenmeyer and add, with shaking, 30 ml HCl (1+1). Heat on steam bath 30 min., shaking flask occasionally to break up any lumps. While cooling inclined flask under tap, add carefully with shaking 30 g KOH pellets at such rate that liquid may boil, but not so violently as to cause loss by spurting. Place flask on steam bath while still hot, cover with small watch glass, and heat 3 hr, swirling mixt. occasionally to carry down any material adhering to sides. Cool until just warm, add 30 ml alcohol and 50 ml H_2O, and mix well. Add 100 ml ether, swirl mixt. vigorously 1 min., and transfer to separator, washing flask with 50 ml and 25 ml portions ether. Wash flask with 50 ml of the KOH soln, pour washings into separator in slow stream while gently swirling liquid, and continue gentle swirling 10–15 sec. Proceed as in **16.015,** beginning "Let liquids sep. (ca 10 min.) . . ." but omitting first acid wash. If emulsion forms and does not break to give sharp interface in 10 min., pour 5 ml alcohol into separator and let stand until sharp interface appears.

Sterols (as Cholesterol)

13.127 *Bromination Method (45)—Official*

Det. sterols in unsaponifiable matter as in **16.018.** However, to unsaponifiable matter from egg-free products or from any product contg <0.23% unsaponifiable matter (as-is basis), add 10 mg cholesterol before applying cholesterol method and correct result accordingly. (For the added cholesterol use highest quality obtainable (m.p. not <147°) and test its purity by carrying 20 mg thru detn.)

13.128 *Digitonin Method (46)—Official*

Weigh 5 g sample, ground to pass at least No. 20 sieve, into 300 ml erlenmeyer and add, with shaking, 15 ml HCl (1+1) in such manner as to keep particles on sides at min. Heat on steam bath 30 min., shaking flask frequently to break up any lumps and insure complete hydrolysis. While cooling inclined flask under tap, add carefully, with swirling, 15 g KOH pellets at such rate that liquid may boil, but not so violently as to cause loss by spurting. Cool, add 20 ml alcohol, rinsing down sides of flask, and heat on steam bath 45 min. with air condenser, shaking frequently.

Add 25 ml H_2O, rinsing down sides of flask, mix well, and cool. Add 50 ml ether, swirl mixt. vigorously 1 min., and transfer to 500 ml separator. Wash flask with 25 and 10 ml portions ether and with 50 ml 1% KOH soln, pouring washings into separator in slow stream while gently swirling liquid, and continue gentle swirling 10–15 sec. Let liquid sep. and slowly drain soap soln into 250 ml separator, but do not drain any small quantity of emulsion or of insol. matter at interface. Rinse down sides of 500 ml separator with 5 ml 1% KOH soln and drain this into smaller separator. Add 25 ml ether to smaller separator and shake vigorously ca 1 min. After liquids sep., discard lower layer. Add ether layer to soln in larger separator, rinsing the 250 ml separator with 10 ml ether. Wash ether soln as before with 3 addnl 50 ml portions 1% KOH, still keeping any insol. matter or emulsion in separator. Wash ether soln twice by swirling with 50 ml H_2O. Finally drain as much of aq. layer as possible without loss of ether soln. Add porcelain chip to 300 ml erlenmeyer, transfer ether to flask, rinse separator with three 5 ml portions ether, and rinse stem of separator with ether. Add rinsings to flask and evap. ether on steam bath.

Dissolve residue in 5 ml acetone; filter, with

suction if necessary, thru Knorr type extn tube contg medium porosity fritted glass disk (Ace Glass, Inc., Vineland, N. J., No. 8571, porosity D, or equiv.), covered with few g washed and ignited sand, into 100 ml centrifuge tube or test tube under bell jar. Wash flask and tube 3 times with 4 ml portions acetone, and rinse tube and stem with few ml acetone (total vol. ca 20 ml). Add 5 ml freshly prepd *digitonin soln in 80% alcohol* contg 40 mg digitonin. (Hasten soln of the digitonin by warming to ca 40–50° under hot H_2O tap). (Products contg >6% egg yolk solids or equiv. (moisture-free basis) require addnl digitonin soln or use of aliquot portion for pptn.) Rotate to mix. Place porcelain chip in the tube, suspend tube in steam bath with small amount of steam to avoid boiling or spattering, evap. nearly to dryness, add 50 ml hot H_2O (near boiling), and stir well with glass rod to disperse ppt and dissolve excess digitonin. Place tube in boiling H_2O bath and hold several min. with frequent stirring. Cool to ca 60°, add 25 ml acetone, mix well by stirring, and cool to room temp. in beaker of cold H_2O.

When ppt has nearly all settled (ca 15 min.), remove glass rod, rinsing off any adhering ppt with acetone. Decant into previously dried and weighed gooch (preferably 10 ml capacity) contg asbestos pad covered with ca 1 g washed and ignited sand. Using wash bottle, wash tube several times with few ml portions acetone to transfer all ppt. (*Caution:* avoid transfer of any particles of chips.) Finally rinse crucible with acetone to dissolve any fat-like material, rinse with 5 ml ether, dry 30 min. at 100°, and weigh. Check wt after second 30 min. of drying. Wt residue $\times 0.243 =$ wt sterol. Report as % sterol on moisture-free basis.

13.129 Extraction, Separation, and Identification of Coloring Matter* (*47*)—Official

Place ca 500 g coarsely ground sample (depending on quantity of color present) in 1 L erlenmeyer, add ca 700 ml 80% alcohol, and shake at intervals for 24 hr, or until no more color is extd. Place in refrigerator overnight to permit dissolved protein to ppt. Filter, and evap. filtrate to 100 ml. Add ca $\frac{1}{4}$ vol. *25% NaCl soln* and slight excess of NH_4OH to filtrate; cool, and transfer to separator. Ext. with equal vol. petr. ether, b.p. <60°; sep. lower layer and repeat extns with addnl portions solvent until no more color is extd. Reserve lower layer, if colored, for further treatment; if colorless, discard.

Combine petr. ether exts and wash with several small portions NH_4OH (1+50) to remove any material mechanically adhering to solvent. This ether soln contains the fats; it also may contain the oil-sol. coal-tar dyes, which can be identified as in (**a**). If colored, immediately acidify alk. aq. soln, freed from fat and oil-sol. coal-tar dyes, with HOAc and ext. in 25 ml portions with two 50 ml portions ether. Solvent, if colored, may contain turmeric, annatto, and trace of saffron, which may be identified as in (**b**).

If original aq. soln, freed from ether-sol. colors, is still colored and H_2O-sol. dyes are suspected, proceed as follows: Ext. aq. soln with 50 ml portions *iso-amyl alcohol* to remove balance of saffron, as well as common orange dyes and Martius yellow; to sep., proceed as in (**c**). Drain lower aq. layer, which, if colored, may contain naphthol yellow S, tartrazine, and sunset yellow. Ext. these dyes with *iso*-amyl alcohol after acidifying soln with HCl to ca $1N$. Remove tartrazine from solvent with $0.25N$ HCl. Sunset yellow is also removed at this stage with slightly lower acid concn, and naphthol yellow S from nearly neutral soln. Proceed as in Chap. **35**.

(**a**) Ext. original petr. ether ext. with two or three 10 ml portions of mixt. consisting of 1 part HCl and 5 parts HOAc.

In presence of yellow OB or yellow AB, pink or red color is obtained. Test small portion of this acid ext. with few drops *40% $SnCl_2$ soln*, which in presence of above dyes causes either decoloration or decided fading. Dil. balance of acid ext. with H_2O, make slightly alk., and ext. color with petr. ether. Wash solvent with two 5 ml portions H_2O to remove excess alkali. Proceed as in Chap. **35**. Remaining coloring matters in petr. ether ext. may be due to natural coloring matter of wheat or coloring matter of egg. Coloring principle of egg yolk, lutein, when heated with *alc. $FeCl_3$*, produces green color. This test, however, is not specific for lutein; carotene and xanthophyll give similar reactions.

(**b**) Wash ether ext. with 5 ml portions H_2O to remove excess acid. To remove annatto and traces of saffron, wash successively with 20 ml portions *5% $NaHCO_3$ soln*. Divide alk. soln into 2 portions. Heat one portion to 60° on steam bath, dye the color on unmordanted cotton, and compare spots tests with a std. Acidify remaining portion of the alk. annatto soln with HOAc and re-ext. with ether. Divide ether ext. into 2 small casseroles and evap. to dryness. Dissolve contents of one casserole in 10 ml NH_4OH (1+9) and impregnate on strip of cotton or filter paper. Orange-yellow to orange-red color is obtained, depending on quantity of dye present. Dry filter paper or cotton, add drop *40% $SnCl_2$ soln*, and again dry. In presence of annatto, purple stain is produced

* See 35.016(b) for corresponding color numbers.

Spot contents of other casserole with H_2SO_4 and HNO_3, which yield blue and greenish-blue colors, resp.

Transfer 2 portions (ca 10 ml each) of original ether ext., from which annatto has been removed, into test tubes and treat with equal vol. 10% NaOH soln and equal vol. HCl (1+1), resp. In presence of turmeric (curcuma) the alk. soln is reddish brown; the acid soln is red.

Turmeric can be further confirmed by its behavior with H_3BO_3. Apply this test as follows: Shake portion of original ether ext. with equal vol. 70% alcohol; to this add $\frac{1}{10}$ vol. HCl, mix, and divide soln equally into 2 test tubes. To one tube add few crystals H_3BO_3 and shake. Use other tube as control. In presence of turmeric, red color is produced after short time.

(**c**) To sep. and identify saffron and the orange coal-tar dyes, dil. the *iso*-amyl alcohol ext. with 2 vols petr. ether and ext. the mixed dyes with several 10 ml portions H_2O. To small portion of this aq. ext. add $\frac{1}{10}$ vol. HOAc and few mg dry *Na hyposulfite* to reduce all the azo dyes. This treatment will not affect the saffron, which can then be re-extd with *iso*-amyl alcohol. After washing solvent repeatedly with small portions of H_2O (to remove decomposition products) evap. to dryness, and confirm presence of saffron by spot tests. Examine remainder of color soln as in Chap. **35**.

13.130 Rapid Method for Tartrazine (*48*)—Official

Place 800 ml cold H_2O and 5 ml NH_4OH in 1 L erlenmeyer and add 200 g unground sample. Stopper flask and shake at intervals; 3–4 hr is usually enough time to disintegrate material. Use glass rod to dislodge material caking on bottom. Centrifuge and decant clear supernatant into 1 L flask. Add soln of 50 g $MgSO_4.7H_2O$ dissolved in 100 ml H_2O, 10 ml *12% silicotungstic acid soln*, and 10 ml HCl; shake well, and let stand 1 hr. (This treatment will ppt almost all protein.)

Centrifuge, decant clear soln into container, and examine as in Chap. **35**.

Carotenoids (*49*)—Official

(Applicable only to detn of carotenoids added for coloring purposes)

13.131 PREPARATION OF STANDARD CURVE

Dissolve 100 mg *natural mixt. of α- and β-carotene* in 5–6 ml CS_2, add 35–40 ml absolute alcohol, cool in refrigerator ca 1 hr to insure max. crystn, and filter on *hard* paper. Dissolve carotene crystals in 5–6 ml CS_2, add 40 ml petr. ether, refrigerate as before, filter on *hard* paper, and dry crystals in vac. desiccator 1 hr.

Weigh accurately 20 mg purified crystals and wash with 20 ml absolute ether into 1 L g-s. vol. flask. Continue to wash with petr. ether, and dil. to vol. by adding petr. ether as soon as carotene dissolves completely. Designate as stock soln.

Prep. 8 concns by adding following quantities of this stock soln to 250 ml vol. flasks: 1.25, 2.50, 3.75, 5.00, 6.25, 7.5, 8.75, and 10.00 ml. Dil. to vol. with petr. ether. These dilns represent concns of 0.10, 0.20, 0.30, 0.40, 0.50, 0.60, 0.70, and 0.80 mg/L. Read these solns at 436 mμ in spectrophotometer or in photometer, using filter No. 44 and 4″ cell. (Cell, filled with solvent, should read zero on photometer scale.) If photometer is used, make 10 readings on each soln and plot av. reading against concn. Obtain line of best fit for data by method of least squares. In applying this method let x represent scale reading and y concn in mg/L; M_x is the mean value of x and M_y is the mean value of y. Substitute in following expressions for values of a and b to give equation $y=a+bx$:

$$b = \frac{\Sigma xy - nM_xM_y}{\Sigma(x)^2 - n(M_x)^2} \quad \text{and} \quad a = M_y - bM_x.$$

Stdze on same day stock soln is prepd

13.132 PREPARATION OF SAMPLE

Grind macaroni and noodles to as near flour fineness as possible in ordinary coffee-type mill. (Products contg egg give no difficulty, but plain macaroni products require several grindings.) Take care not to set mill too tight, as enough heat may be generated to damage pigments.

13.133 REAGENTS

(**a**) *Alcoholic potassium hydroxide soln.*—Dissolve 10 g KOH in 100 ml alcohol by warming on steam bath.

(**b**) *Methanol.*—92%. 8 ml H_2O+92 ml absolute MeOH.

(**c**) *Adsorption mixture.*—Mix equal portions by vol. of activated magnesia (Micron brand No. 2641, 2642, or SeaSorb 43, Westvaco Products Co., Newark, Calif.) and diatomaceous earth (Hyflo Super-Cel, Johns-Manville, 22 W. 40th St., New York, N. Y.).

13.134 DETERMINATION OF TOTAL CAROTENOIDS AND CAROTENE

Weigh 20 g flour, semolina, or macaroni, or 10 g egg noodles, or 2 g egg yolk into 125 ml erlenmeyer. Add 50 ml of the alc. KOH soln and boil on steam bath 30 min. under reflux condenser. Rotate flask occasionally but be as careful as possible to keep sample from collecting on sides of flask. Remove flask and cool to room temp. Filter thru büchner-type medium fritted glass filter into

250 ml suction flask, using suction, transferring most of material with few ml alcohol from wash bottle. Turn off suction, rinse flask with 25 ml ether, pour rinsing onto glass filter, and stir material with rod to allow ether to come in contact with all portions. Filter, and repeat this procedure twice.

Transfer filtrate to 250 ml g-s. separator and rinse with ca 25 ml ether, disregarding soapy material in flask. Add 175 ml H_2O, carefully invert, and rotate several times. When layers sep., remove lower aq.-alcohol layer and ext. this layer again with 25 ml ether. Discard lower layer and add the ether to original ether soln. Wash ether by pouring 50 ml H_2O thru it. After layers sep., withdraw aq. layer and discard. Add 50 ml petr. ether to ether soln, and wash with five 50 ml portions H_2O, carefully inverting and rotating separator. Discard all aq. layers (slight emulsions usually clear in few min. but may be discarded, especially if there is no significant yellow color.)

Transfer ether-petr. ether mixt. to 250 ml distn flask, rinsing separator with petr. ether; place flask in beaker of H_2O at 45–50°. Stopper flask, connect side arm with vac., and conc. to ca 5 ml to remove ether. Filter thru Allihn type absorption tube with coarse fritted glass plate contg ca $\frac{3}{8}''$ layer anhyd. powd. Na_2SO_4, or thru 5.5–7.0 cm paper half filled with the Na_2SO_4 (use small, long-stem funnel reaching thru neck of flask) into 25 vol. flask (100 ml for neutral wedge photometer). Dil. to vol. with petr. ether used to rinse distn flask and which has been passed portionwise thru the filter contg Na_2SO_4, and mix by inverting few times. Transfer to 1 cm absorption cell and read absorbance at 436 mμ in spectrophotometer, making at least 3 readings. Calc. from av. reading the total carotenoid pigment in ppm from std curve. If pure carotene is not available for stdzn, multiply absorbance by 64.2 for yolk, 13.05 for noodles, or by 6.52 for semolina and macaroni.

13.135 SEPARATION OF CAROTENE FROM XANTHOPHYLLS

(a) *Carotene by phase separation.*—Transfer all soln from cell and vol. flask quantitatively to 125 ml separator, rinse with petr. ether, and dil. to ca 100 ml vol. Add 15 ml 92% MeOH (by vol.), shake moderately ca 2 min. by hand or 10 min. on mechanical shaker, and let separator stand in upright position ca 1 min. until layers sep. Decant lower layer contg xanthophyll and repeat extns 5 more times or until aq. MeOH layer is nearly colorless for semolina. (Eight extns are generally enough for noodles but higher than normal egg content may require 10 extns.) Examine final MeOH layer recovered in test tube over white background to be sure soln is nearly colorless.

Wash petr. ether with 25 ml H_2O, inverting separator several times; discard aq. layer and repeat twice more. Filter petr. ether thru Allihn type absorption tube contg $\frac{1}{4}''$ layer of anhyd. powd. Na_2SO_4, or thru 9 cm paper half filled with the Na_2SO_4, into 250 ml distn flask, washing color from filter with petr. ether. Conc. to 5 ml by vac. as in **13.134** and transfer to 10 ml vol. flask, using very small portions of petr. ether; dil to vol., mix by inverting, and read absorbance in spectrophotometer as in **13.134.** (For neutral wedge photometer dil. to 25–50 ml vol., depending on concn of carotene.) Calc. carotene in ppm from std curve. If pure carotene is not available for stdzn, multiply absorbance by 5.22 for noodles, or by 2.61 for semolina.

(b) *Carotene by chromatographic separation.*—Prep. column in absorption tube of ca 18 mm o.d.×240 mm with ca 5 cm tip inserted thru rubber stopper. Loosely plug with small pad of cotton, place in 250 ml suction flask, and turn on suction. Add absorption mixt., **13.133**(c), thru funnel in small amounts from spatula to height of ca 11 cm; pack column by pressing down (*only once after all this mixt. has been added*) with cork stopper, just fitting the tube, on end of rod. Place on top 1–2 cm anhyd. powd. Na_2SO_4.

Transfer all soln from cell and vol. flask quantitatively to 250 ml distn flask, and conc. as in **13.134** to ca 5 ml, continuously applying suction to flask. Transfer to absorption tube and rinse with four ca 5 ml portions petr. ether to remove all color. Finally rinse down sides of tube with few ml petr. ether. After few drops have come thru absorption tube, change to another 250 ml suction flask. When nearly all petr. ether is down to Na_2SO_4 layer, add 50 ml petr. ether-acetone mixt. (9+1) to wash thru carotene. When all this solvent has passed thru the Na_2SO_4, turn off suction. (Keep top of column covered with solvent during entire operation.) Transfer carotene soln (which should be only few ml) to 10 ml vol. flask, using very small portions of petr. ether, dil. to vol. with petr. ether, and mix by inverting. Read as under (a) and calc. in ppm. (These solns should be read on same day as extn.)

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(*45*) Ibid. **24**, 75, 143(1941); **25**, 83, 639(1942).

(*46*) Ibid. **37**, 92, 408(1954).

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14. Coffee and Tea

GREEN COFFEE

14.001 Macroscopic Examination—Procedure

Macroscopic examination usually shows presence of excessive quantities of black and blighted coffee beans, coffee hulls, stones, and other foreign matter. Sep. these by hand picking and det. quantity by wt.

14.002 Coloring Matters—Procedure

Shake vigorously 100 g or more of sample with cold H_2O or alcohol, 70% by vol. Strain thru coarse sieve and let settle. Identify sol. colors in soln and insol. pigments in sediments as in Chap. 35.

Chlorogenic Acid (*1*)—First Action

14.003 APPARATUS

(a) *Grinder.*—Burr mill, impact mill, or hammer mill; Labconco Laboratory Mill, Mikro-Samplmill, Raymond Laboratory Mill, or equiv.

(b) *Glass stirrers.*—Mechanical stirrer with glass rods with paddle-shape blade at lower end reaching bottom of 200 ml Pyrex Kohlrausch or vol. flask.

(c) *Spectrophotometer.*—Instrument capable of measuring absorbance at 324 mμ, such as Beckman Model DU.

14.004 REAGENTS

(a) *Basic lead acetate soln.*—Sp. gr. 1.25. Soln **29.146** may be used, or prep. soln from dry powder or Horne's Dry Lead.

(b) *Chlorogenic acid.*—Prep. from green coffee as follows: Defat 100 g green coffee, ground to 30 mesh, on büchner with three 250 ml portions petr. ether. Air-dry until odor of petr. ether is no longer detected. Place residue in 2 L erlenmeyer, add 1 L boiling H_2O, and boil gently 15 min. (Boil carefully, as soln tends to froth.) Filter hot soln with suction thru large büchner, using filter aid if necessary. Add, with stirring, 100 ml basic $Pb(OAc)_2$ soln and cool to room temp. Collect lemon-yellow ppt by centrifuging, and discard supernatant. Wash 4 times by suspending ppt in 100 ml H_2O and centrifuging. Suspend ppt in H_2O, dil. to ca 400 ml, and add 10% $HClO_4$, with vigorous stirring, to pH 1.7. Centrifuge to remove insol. residue and discard. Reppt Pb chlorogenate by adding 10% NaOH soln to pH 4.1. Collect ppt and wash with 100 ml portions H_2O as before. Suspend in H_2O and dil. to ca 200 ml. Pass in H_2S with vigorous stirring 20 min., and filter thru büchner. Sat. filtrate with NaCl and ext. in separator with eight 50 ml portions EtOAc. Dry combined EtOAc exts ca 12 hr with 50 g anhyd. Na_2SO_4. Filter and distill off solvent until liquid just begins to turn cloudy or reaches vol. of 100 ml. Cool, and add 2 vols petr. ether. Collect fluffy ppt on büchner, wash with petr. ether, and air-dry until no petr. ether odor remains. Recrystallize twice from H_2O, decolorizing with activated C, if necessary. M. p. of product after drying in vac. over efficient desiccant is 207–209°. Absorptivity is not <52.0.

14.005 PREPARATION OF SAMPLE SOLUTION

Rough-grind 100 g whole beans, green or roasted, to pass No. 10 sieve. Mix thoroly and grind 10 g sample of the ground material or pre-ground roasted coffee to pass No. 30 sieve, regrinding any oversize particles until entire sample passes sieve.

Green coffee.—Weigh 0.7 g ground sample into 50 ml centrifuge tube. Add 25 ml petr. ether, mix thoroly, centrifuge, and decant supernatant. Repeat twice. Dry residue in gentle stream of air until odor can no longer be detected. Transfer to 750 ml erlenmeyer with small amount of H_2O. Add 400 ml boiling H_2O, reheat quickly to boiling, and continue to boil gently exactly 15 min.; then cool quickly to room temp. under tap. During boiling, swirl flask frequently to keep coffee submerged in soln. Transfer to 500 ml vol. flask and dil. to vol. Filter thru retentive paper, discarding first 25–50 ml filtrate. If filtrate is more than faintly cloudy, refilter thru fine porosity fritted glass filter, using suction. Do not use filter aids.

14.006 DETERMINATION

Transfer 10 ml filtrate to 100 ml vol. flask and dil. to vol. with H_2O. Det. absorbance at 324 mμ against H_2O. Transfer 100 ml sample soln to 200 ml Pyrex vol. flask or Kohlrausch flask. Add 2 ml *satd KOAc* soln and 10 ml basic $Pb(OAc)_2$ soln with swirling. Place flask in boiling H_2O bath 5 min., swirling occasionally. Remove, cool under tap, and place in ice-H_2O bath. Stir mechanically 1 hr with flask immersed in bath. Remove, wash down stirrer, warm to room temp., and dil. to vol. with H_2O. Filter thru fluted paper, discarding first 25–50 ml filtrate. Det. absorbance of soln at 324 mμ immediately. Clean cells carefully after each

use of Pb-treated solns because $PbCO_3$ slowly accumulates on optical surfaces.

From std curve det. (1) apparent concn of chlorogenic acid in soln taken for absorbance measurement without Pb treatment (C_0); (2) apparent concn in filtrate after Pb treatment (C_1). From latter value subtract 0.00045 mg/ml to correct for solubility of Pb chlorogenate.

Calc. corrected concn $= C_0 - [(C_1 - 0.00045)/5]$.

14.007 PREPARATION OF STANDARD CURVE

Weigh 40.0 mg dried chlorogenic acid, transfer to 500 ml vol. flask, dissolve, and dil. to vol. with H_2O. Prep. series of stds by transferring 5, 10, 15, and 20 ml aliquots to 100 ml vol. flasks and dilg to vol. Det. absorbance at 324 mμ of each soln against H_2O. Plot concn of chlorogenic acid in mg/ml against absorbance.

ROASTED COFFEE

14.008 Macroscopic Examination—Procedure

Pick out and identify microscopically artificial coffee beans, apparent from their regular form, and roasted legumes and lumps of chicory in whole roasted coffee. For ground coffee, sprinkle some of sample on cold H_2O and stir lightly. Fragments of pure coffee float, if not overroasted, while fragments of chicory, legumes, cereals, etc., sink immediately, chicory coloring the H_2O decidedly brown. In all cases use microscopic examination to identify particles that sink.

14.009 Preparation of Sample—Official

Grind sample to pass thru No. 30 sieve and store in tightly stoppered bottle.

14.010 Loss on Drying—First Action

Dry 5 g sample to constant wt at temp. of boiling H_2O under pressure not >100 mm Hg, or at 105–110° under atmospheric pressure, 5 hr and subsequent periods of 1 hr each. For whole coffee, grind rapidly to coarse powder, and without sifting and unnecessary exposure to air weigh portions for detn. For ground coffee, sample directly without further grinding.

14.011 Soluble Solids—Official

Place 4 g prepd sample, **14.009**, in 200 ml vol. flask. Add H_2O to mark, let infuse 8 hr, with occasional shaking, and let stand 16 hr longer without shaking. Filter and evap. 50 ml filtrate to dryness in flat-bottom dish. Dry at 100°, cool, and weigh.

14.012 Ash—Official

Proceed as in **29.012** or **29.013**, using sample prepd as in 14.009.

14.013 Soluble and Insoluble Ash—Official

Proceed as in **29.015**, using the ash obtained in **14.012**.

14.014 Alkalinity of Soluble Ash—Official

Proceed as in **29.016**, using the filtrate obtained in **14.013**.

14.015 Ash Insoluble in Acid—Official

Proceed as in **28.005**, using the ash obtained in **14.012** or H_2O-insol. ash obtained in **14.013**.

14.016 Soluble Phosphorus in the Ash—Official

Proceed as in **2.019** or **2.022**, using soln obtained in **14.013**.

14.017 Insoluble Phosphorus in the Ash—Official

Boil insol. ash, **14.013**, with 25 ml HCl $(1+2)$, filter, wash thoroly with hot H_2O, and det. P_2O_5 in combined filtrate and washings as in **2.019** or **2.022**.

14.018 Chlorides—Official—*See* 6.065 and 6.066

Caffeine

14.019 *Power-Chesnut Method (2)—Official*

(Not applicable to coffee exts)

Moisten 10 g prepd sample, **14.009**, with alcohol, transfer to Soxhlet or similar extn app., and ext. with alcohol 8 hr, taking care to insure complete extn. Transfer ext. with hot H_2O to porcelain dish contg 10 g *heavy MgO* suspended in 100 ml H_2O. Evap. slowly on steam bath with frequent stirring to dry, powdery mass. Rub residue with pestle into paste with boiling H_2O and transfer with hot H_2O to smooth filter, cleaning dish with policeman. Collect filtrate in 1 L flask marked at 250 ml and wash with boiling H_2O until filtrate reaches mark. Add 20 ml H_2SO_4 $(1+9)$ and boil gently 30 min., with funnel in neck of flask. Cool, filter thru moistened double paper into separator, and wash with small portions H_2SO_4 $(1+199)$.

Ext. with six 25 ml portions $CHCl_3$. Wash combined $CHCl_3$ exts in separator with 5 ml 1% KOH soln. Filter the $CHCl_3$ into erlenmeyer. Wash the KOH soln with two 10 ml portions $CHCl_3$, adding them to flask together with $CHCl_3$ washings of filter paper. Evap. or distill on steam bath to small vol. (10–15 ml), transfer with $CHCl_3$ to weighed beaker, evap. carefully, dry

30 min. at 100°, and weigh. To test purity of residue det. N and multiply by factor 3.464.

With products very low in caffeine, combine caffeine residues from duplicate detns (representing 20 g original material) and det. N as in **2.036**, using half the quantity of reagents specified for digestion. Steam out app. thoroly before distg. Distill to small vol. in distg flask to insure removal of all NH_3. Correct for blank obtained, using same reagents and app., and pure sucrose in place of caffeine.

14.020 *Bailey-Andrew Method—Official*

Proceed as in **14.044**, using 50 g decaffeinated coffee, 25 g decaffeinated sol. coffee, 10 g regular coffee, or 5 g regular sol. coffee.

14.021 *Micro Bailey-Andrew Method (3)—First Action*

Weigh 5 g decaffeinated coffee (2 g decaffeinated instant coffee, 2 g regular coffee, or 1 g regular instant coffee), add 5 g powd. MgO, and transfer to weighed 500 ml erlenmeyer. Add ca 150–200 ml H_2O, heat to boiling, and boil 45 min., shaking occasionally. Add H_2O, when necessary, to prevent frothing (final wt of H_2O must be 100 g). Cool to room temp. Make mixt. to tare wt+105 g+sample wt.

Filter sample directly into 50 ml graduate until exactly 50 ml soln (equiv. to $\frac{1}{2}$ sample wt) is obtained. Transfer soln to 125 ml separator. Wash graduate with 2 ml H_2O and add washing to separator. Add 4 ml H_2SO_4 (1+9). Ext. with five 10 ml portions $CHCl_3$, shaking vigorously 1 min. for each extn. Let emulsion break; then drain $CHCl_3$ into 125 ml separator. Add 5 ml 1% KOH soln. Shake vigorously 1 min., let emulsion break, and drain $CHCl_3$ thru cotton plug into 100 ml Kjeldahl flask. Ext. KOH soln with 5 ml $CHCl_3$ and add to Kjeldahl flask. To digestion flasks add 1.30 ± 0.5 g K_2SO_4 and 40 ± 5 mg HgO. Add boiling chips and rinse down neck of flask with 3 ml $CHCl_3$. Place flask on digestion rack and conc. $CHCl_3$ to ca 20 ml. Then proceed as in **38.011**, using $0.01N$ acid. 1 ml $0.01N$ acid = 0.485 mg caffeine.

14.022 **Chlorogenic Acid (*1*)—First Action**

(a) *Roasted coffee.*—Weigh 1 g ground sample, transfer to 750 ml erlenmeyer, and proceed as in **14.005**, beginning "Add 400 ml boiling H_2O, . . . "

(b) *Instant coffee.*—Weigh 0.35 g sample, transfer to 500 ml vol. flask, dil. to vol., and proceed as in **14.006**.

14.023 **Crude Fiber—Official**

Proceed as in **22.040**, using sample prepd as in **14.009**.

14.024 **Starch—Official**

Extract 5 g prepd sample, **14.009**, on hardened filter with five 10 ml portions ether, wash with small portions alcohol until total of 200 ml has passed thru, and proceed as in **22.045**, beginning with second sentence.

14.025 **Sugars (*4*)—Official**

Weigh 10 g prepd sample, **14.009**, into 250 ml vol. flask, add 1 g powd. NH_4NaHPO_4, and proceed as in **22.041** and **22.042**. Det. Cu in Cu_2O ppt either volumetrically, **29.042**, or electrolytically, **29.045**.

14.026 **Petroleum Ether Extract—Official**

Dry 2 g prepd sample, **14.009**, at 100°, ext. with petr. ether (b.p. 35–50°) 16 hr, evap. solvent, dry residue at 100°, cool, and weigh.

14.027 **Total Acidity—Official**

Treat 10 g prepd sample, **14.009**, with 75 ml alcohol, 80% by vol., in erlenmeyer, stopper, and let stand 16 hr, shaking occasionally. Filter, transfer aliquot of filtrate (25 ml for green coffee, 10 ml for roasted coffee) to beaker, dil. to ca 100 ml with H_2O, and titr. with $0.1N$ alkali, using phthln. Express result as ml $0.1N$ alkali required to neutralize acidity of 100 g sample.

14.028 **Coating and Glazing Substances—Procedure**

(a) *Sugar and dextrin.*—To 100 g whole coffee in beaker, add exactly 300 ml H_2O, stir, and let stand 5 min., stirring frequently. Filter thru dry paper and carefully add dry $Pb(OAc)_2$ to filtrate until pptn is complete, avoiding excess reagent. Filter thru dry filter and remove Pb from filtrate by adding slight excess of dry, powd. $K_2C_2O_4$. Filter thru dry filter and det. reducing sugars as invert sugar in 50 ml of the filtrate as in **29.039**.

Invert 75 ml aliquot filtrate as in **29.026**(b). Cool, nearly neutralize with NaOH soln (1+1), dil. to 100 ml, and det. reducing sugars as invert sugar in resulting soln as in **29.039**.

Measure 100 ml aliquot filtrate into 200 ml vol. flask, add 10 ml HCl (sp. gr. 1.125), and hydrolyze as in **22.043**. Cool, neutralize with NaOH soln (1+1), dil. to vol., filter thru dry filter, and det. reducing sugars as invert sugar in 50 ml filtrate as in **29.039**.

Calc. reducing sugars in each instance to % by wt original coffee. Calc. sucrose from reducing sugars before and after inversion as in **29.032**, and calc. dextrin as follows: Subtract reducing sugars after inversion from reducing sugars after hydrolysis and multiply difference by factor 0.8605.

In some instances presence of sucrose in H_2O ext. may be verified by polarization. Presence of

dextrin in H_2O ext. may be verified by polarization as in **29.034**, and by erythrodextrin test, (**b**).

(**b**) *Erythrodextrin test for commercial glucose.*—To aq. ext., (**a**), prior to clarification with $Pb(OAc)_2$, add few ml I soln (1 g I, 3 g KI, 50 ml H_2O). In presence of commercial glucose, soln turns red or violet, depth and character of color depending upon quality and nature of glucose used. If amount of glucose is very small, ppt dextrin that may be present by adding several vols alcohol. Let ppt settle (do not filter), decant liquid, dissolve residue of dextrins in hot H_2O, cool, and apply I test. Negative result does not prove absence of commercial glucose, because some glucose, especially of high conversion, does not give reaction with I.

(**c**) *Egg albumen and gelatin.*—Add 500 ml H_2O to 100 g whole coffee and let stand 5 min., stirring frequently. Filter and treat sep. portions of filtrate with (1) *5% soln of tannic acid*, and (2) Millon reagent, **22.014.** Boil third portion of filtrate. In presence of egg albumen more or less heavy ppt will form in each case.

As confirmatory test, treat aliquot of filtrate with excess tannic acid soln, add little NaCl if necessary to secure flocculation of ppt, filter, and without washing, insert paper and contents into Kjeldahl flask and det. N. By this method coffee not coated with albumen or gelatin yields <10 mg N/100 g sample.

(**d**) *Fats and waxes.*—Treat 100–200 g coffee beans 10 min. with low-boiling petr. ether, pour off petr. ether, and repeat process. Filter combined ext., evap., and det. refractive index and saponification number of the residue as in **26.007** and **26.023.**

14.029 Chicory Infusion—Procedure

Cover 100–150 g whole coffee with H_2O, let soak 2–3 min., stirring frequently, and drain aq. washings thru coarse sieve. Wash coffee on sieve with ca 100 ml H_2O and centrifuge combined washings. Decant clear liquid from sediment, drain sediment almost dry on filter paper, mount in chloral hydrate soln, **28.025(b)**, and examine under microscope for chicory.

TEA

14.030 Preparation of Sample—Official

Grind sample to pass thru No. 30 sieve.

14.031 Moisture—Official—*See* 22.003

14.032 Water Extract (*5*)—Official

To 2 g ground sample in 500 ml vol. flask, add 200 ml hot H_2O and boil over low flame 1 hr, rotating occasionally. Close flask with rubber stopper thru which passes glass tube 30″ long for condenser. Boil very slowly so that no steam escapes from top of air condenser. Cool, dil. to vol., mix thoroly, and filter thru dry paper. Transfer 50 ml aliquot to weighed dish and evap. to dryness on steam bath. Place in oven, heat 1 hr at 100°, cool, and weigh.

14.033 Ash—Official—*See* 29.012 or 29.013

14.034 Soluble and Insoluble Ash—Official

Proceed as in **29.015,** using the ash obtained in **14.033.**

14.035 Alkalinity of Soluble Ash—Official

Proceed as in **29.016,** using the filtrate obtained in **14.034.**

14.036 Alkalinity of Insoluble Ash—Official

Proceed as in **29.017,** using insol. ash obtained in **14.034.**

14.037 Ash Insoluble in Acid—Official

Proceed as in **28.005,** using total ash obtained in **14.033,** or insol. residue obtained in **14.034.**

14.038 Soluble Phosphorus in the Ash—Official

Proceed as in **2.019** or **2.022,** using soln of sol. ash obtained in **14.035.**

14.039 Insoluble Phosphorus in the Ash—Official

Proceed as in **2.019** or **2.022** using soln obtained in **14.036.**

14.040 Petroleum Ether Extract—Official—*See* 14.026

14.041 Protein—Official

Det. N as in **2.036.** Protein = (% total N − % N present as caffeine) ×6.25.

14.042 Crude Fiber—Official—*See* 22.040

Caffeine

14.043 *Power-Chestnut Method (6)* *Official—See* 14.019

14.044 *Modified Bailey-Andrew Method (7)—Official*

Into weighed 1 L erlenmeyer weigh 5 g prepd sample, **14.030.** Add ca 500 ml H_2O, swirl, and heat to boiling. Add 10 g heavy MgO. Boil gently over low flame 2 hr with occasional shaking. Add

H_2O to prevent frothing and to wash down sides of flask. Cool, and make to wt with H_2O (tare wt + 510 g + wt sample). Filter, collect 200 ml clear filtrate (equiv. to 0.4 sample wt), add 20 ml H_2SO_4 (1+9), and transfer to 500 ml separator. Shake 6 times with $CHCl_3$, using 25, 20, 15, 10, 10, and 10 ml portions. Treat combined exts with 5 ml 1% KOH soln; when liquids sep. completely drain $CHCl_3$ layer into Kjeldahl flask. Wash alk. soln in separator with two 10 ml portions $CHCl_3$ and combine washings with remaining bulk of ext. Evap. or distill off the $CHCl_3$ to <25 ml, and proceed as in **2.036**. 1 ml $0.1N$ H_2SO_4 = 4.85 mg anhyd. caffeine. For materials low in caffeine, use $0.04N$ reagents.

Tannin (*8*)—Official

14.045 REAGENTS

(**a**) *Potassium permanganate soln.*—Prep. soln contg 1.33 g/L and obtain its equiv. of $0.1N$ oxalic acid.

(**b**) *Indigo carmine soln.*—Prep. soln contg 6 g indigo carmine (free from indigo blue) and 50 ml H_2SO_4/L.

(**c**) *Gelatin soln.*—Soak 25 g gelatin 1 hr in satd NaCl soln, heat until gelatin dissolves, cool, and dil. with satd NaCl soln to 1 L.

(**d**) *Acid sodium chloride soln.*—Acidify 975 ml satd NaCl soln with 25 ml H_2SO_4.

14.046 DETERMINATION

Boil 5 g sample 30 min. with 400 ml H_2O, cool, transfer to 500 ml vol. flask, and dil. to mark. To 10 ml of the infusion (filtered, if not clear) add 25 ml of the indigo carmine soln and ca 750 ml H_2O. Add the $KMnO_4$ soln from buret, little at time while stirring, until color becomes light green; then dropwise until color changes to bright yellow or to faint pink at rim. Designate ml $KMnO_4$ used as *a*.

Mix 100 ml of the clear infusion of tea with 50 ml of the gelatin soln, 100 ml of the acid NaCl soln, and 10 g *powd. kaolin*, and shake several min. in stoppered flask. Let mixt. settle and decant thru filter. Mix 25 ml filtrate with 25 ml of the indigo carmine soln and ca 750 ml H_2O, and titr. with $KMnO_4$ as before. Ml $KMnO_4$ used subtracted from that obtained above, *a*, gives quantity of $KMnO_4$ required to oxidize the tannin. 1 ml $0.1N$ oxalic acid = 0.0042 g tannin (gallotannic acid).

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(*3*) Ibid. **40,** 346(1957); **43,** 620(1960).
(*4*) J. Assoc. Offic. Agr. Chemists, **3,** 498(1920).
(*5*) Ibid. **7,** 154(1923).
(*6*) J. Am. Chem. Soc. **41,** 1298(1919); J. Assoc. Offic. Agr. Chemists **5,** 288(1921); **6,** 107(1922).
(*7*) J. Assoc. Offic. Agr. Chemists **5,** 291(1921); **6,** 107(1922); **43,** 620(1960).
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15. Dairy Products

MILK

15.001 COLLECTION OF SAMPLE—PROCEDURE

Sample size necessary varies with analyses required. For usual analysis collect 250–500 ml (½–1 pint) sample; for fat detn only, collect 50–60 ml (ca 2 fl oz).

For bottled milk collect one or more containers as prepd for sale. Thoroly mix bulk milk by pouring from one clean vessel into another 3 or 4 times or stir at least 30 sec. with utensil reaching to bottom of container. If cream has formed, detach all of it from sides of vessel and stir until liquid is evenly emulsified or use hand homogenizer.

Place in non-absorbent, air-tight containers and keep cold, but above freezing temp., until examined. When transporting samples, completely fill containers, stopper tightly, and identify. Tablets contg $HgCl_2$, $K_2Cr_2O_7$, or other suitable preservative, at least 0.5 g active ingredient per tablet for each 8 fl oz milk, but total wt of such tablet not >1 g, or 36% soln of HCHO, 0.1 ml (2 drops) per fl oz, may be used unless presence of preservative is objectionable in physical or chemical tests to be made in addn to detn of fat. If phosphatase test is to be made, only $CHCl_3$ can be used as preservative, and stoppers must be of phenol-free material such as red rubber.

15.002 PREPARATION OF SAMPLE—PROCEDURE

Bring sample to ca 20°, mix until homogeneous by pouring into clean receptable and back repeatedly, and promptly weigh or measure test portion. If lumps of cream do not disperse, warm sample in H_2O bath to ca 38° and keep mixing until homogeneous, using policeman, if necessary, to reincorporate any cream adhering to container or stopper. Where practical and fat remains dispersed, cool warmed samples to ca 20° before transferring test portion.

When Babcock method, **15.031**, is used, adjust both fresh and composite samples to ca 38°, mix until homogeneous as above, and immediately pipet portions into test bottles.

15.003 **Specific Gravity—Procedure**

Det. sp. gr. at 15.6/15.6° with pycnometer or std hydrometer.

15.004 **Acidity (*1*)—Official**

Measure or weigh suitable quantity (ca 20 ml or 20 g) sample into suitable dish and dil. with twice its vol. CO_2-free H_2O. Add 2 ml phthln, and titr. with $0.1N$ NaOH to first persistent pink. If measured vol. sample was used, det. its wt from sp. gr. of sample. Report acidity as % lactic acid by wt. (1 ml $0.1N$ NaOH $=0.0090$ g lactic acid.) If Babcock milk pipet, **15.030(b)**, is used, ml $0.1N$ NaOH required $\div 20 = \%$ acid as lactic acid.

Results may also be expressed as ml $0.1N$ NaOH/100 g sample.

Citric Acid (*2*)—Official

15.005 PREPARATION OF SAMPLE

To 50 g milk in 150 ml beaker, add ca 100 mg *tartaric acid* and 6 ml $1N$ H_2SO_4 and heat on steam bath 15 min. Immediately add 3 ml *20% phosphotungstic acid* soln, mix well, and return to steam bath for 5 min. Transfer to 250 ml vol. flask with alcohol, cool, dil. to mark with alcohol, mix, and filter thru folded paper. Pipet 200 ml clear filtrate into centrifuge bottle.

15.006 REAGENTS

Use reagents specified in **20.046.**

15.007 DETERMINATION

To soln in centrifuge bottle add 10 ml of the $Pb(OAc)_2$ soln, shake vigorously ca 2 min., and centrifuge at ca 1000 rpm 15 min. Carefully decant supernatant from pptd Pb salts and test with little $Pb(OAc)_2$ soln. If ppt forms, return to centrifuge bottle, add more Pb soln, shake, and again centrifuge. If sediment lifts, centrifuge again, increasing speed and time, and decant. Invert bottle and drain thoroly for several min. To Pb salts in centrifuge bottle add ca 150 ml H_2O, shake thoroly, and sat. with *H_2S*. Transfer to 250 ml vol. flask, dil. with H_2O to mark, mix, and filter thru folded paper. Proceed as in **20.049.**

Lactic Acid (*3*)—Official

15.008 PREPARATION OF SOLUTION

(a) *Liquid, whole, and skim milks.*—Weigh 50 g into 100 ml vol. flask.

(b) *Dried, whole, and skim milks.*—Weigh 5 g into 100 ml beaker, and using heavy stirring rod,

make into smooth paste with H_2O. Transfer contents of beaker to 100 ml vol. flask with ca 50 ml H_2O.

(c) *Cream and ice cream.*—Weigh 20 g into 100 ml vol. flask and add ca 50 ml H_2O.

(d) *Sweetened condensed milk.*—Weigh 25 g into 100 ml beaker and transfer to 100 ml vol. flask with ca 50 ml H_2O.

(e) *Evaporated milk.*—Weigh 25 g into 100 ml vol. flask and add ca 50 ml H_2O.

To mixts add 6 ml 1*N* H_2SO_4 and mix, avoiding vigorous agitation. Add 5 ml *20% phosphotungstic acid soln* (1 ml for cream and 2 ml for ice cream) and dil. to mark with H_2O. Mix, and filter thru folded paper.

(f) *Butter.*—Weigh 20 g into centrifuge bottle, add 25 ml H_2O, and warm on steam bath. Neutralize contents of bottle with 1*N* NaOH, using phthln. Cool, add 50 ml ether, and mix well, avoiding violent agitation. Add 50 ml petr. ether, mix well, and centrifuge. Draw off ether layer as completely as possible by siphon with lower end bent upward. Repeat extn, using 25 ml of each of the ethers. Place bottle on steam bath to remove most of remaining ether. Transfer residue in bottle to 100 ml vol. flask, add 3 ml 1*N* H_2SO_4, and mix. Cool mixt. and ppt proteins with *20% phosphotungstic acid soln*, adding reagent dropwise until pptn stops. Dil. to mark, mix by shaking, and filter thru folded paper.

15.009 REAGENTS

(a) *Barium lactate std soln.*—Dissolve in ca 10 ml H_2O quantity of a pure lactate, such as Li, Zn, or Ca lactate, contg equiv. of ca 300 mg free lactic acid. Transfer material to extractor, Fig. 26, add 0.5 ml H_2SO_4 (1+1), and adjust vol. to 50 ml. Ext. with ether 3 hr. Add ca 20 ml H_2O to extn flask, evap. ether on steam bath, and carefully titr. soln with 0.1*N* $Ba(OH)_2$. Transfer neutralized material to 200 ml vol. flask, dil. to mark, and mix. Pipet into 500 ml vol. flask quantity of this Ba lactate soln contg equiv. of exactly 250 mg free lactic acid, dil. to mark, mix, and designate as *lactate std soln.* (2 ml equiv. to 1 mg lactic acid. To plot std curve use freshly prepd soln.) Transfer 20 ml of the lactate std soln to 100 ml vol. flask, dil. to mark, and designate as *dil. std lactate soln* (10 ml equiv. to 1 mg lactic acid).

(b) *Carbon.*— To 10 g high-grade C (Nuchar W, Suchar, Darco G60, or Carbex E) in 600 ml beaker, add ca 200 ml H_2O and 30 ml 1*N* HCl, and keep on steam bath 20 min., agitating continuously with air passed thru cotton. Filter on büchner and suck as dry as possible, tamping with flat-end rod. Transfer cake to beaker, add ca 200 ml H_2O, mix thoroly, and refilter. Repeat washing and filtering twice, and dry at 100°.

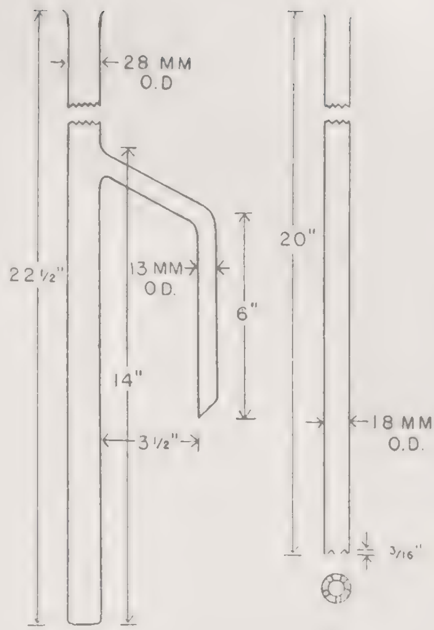

FIG. 26.—LIQUID EXTRACTOR

(c) *Ferric chloride soln.*— Dissolve 2 g $FeCl_3 \cdot 6H_2O$ in H_2O, add 5 ml 1*N* HCl, and dil. to 200 ml.

15.010 PREPARATION OF STANDARD CURVE

Transfer from buret to vol. flasks, graduated at 50 and 55 ml, quantities of std solns in left-hand column of **15.011.** Right-hand column gives mg of lactic acid in the 40 ml filtrate from each sample after C treatment described below, and that will therefore be read in photometer. Blank using 40 ml H_2O in place of lactate soln must be included in each series.

To each flask add 6.6 ml 0.1*N* HCl and H_2O until vol. is ca 40 ml. Now add 200 ± 1 mg of the prepd C, shake, and keep on steam bath 10 min., mixing frequently. Cool, dil. to 55 ml mark with H_2O at room temp., and promptly filter thru quant. paper, pouring back until clear.

Transfer 40 ml of each clear filtrate to 50 ml Nessler tube. As the 40 ml filtrate used contains only 4.8 ml of the acid added during the C treatment, add 1.2 ml 0.1*N* HCl. (Total of 6 ml 0.1*N* HCl is required in the tube.) Place each tube in jacket of black paper. Pipet 5 ml of the $FeCl_3$ soln into one tube at time, dil. to mark, and mix. Pour soln into 4″ photometer cell (preferably Pyrex) with plane parallel fused ends, and with side walls painted black; read in neutral wedge photometer, using filter No. 46. (Other types of photometers may be used. On exposure to direct light, color fades, but protected as provided it is stable for number of hr.) From readings obtained, prep. std curve, plotting mg lactic acid as abscissa and

scale readings as ordinates. (Large-scale graph paper is recommended to permit more accurate interpolations.)

It is not necessary to prep. new std curve when new batch of C or soln of $FeCl_3$ is used. However, blank detn with H_2O should be made, and if this blank does not coincide with original blank, bring readings into conformity with curve by adding to or subtracting from readings the observed variance of new blank from old blank.

15.011 *Prepn of dilns for std curve*

Soln to be transferred to 50–55 ml vol. flask	Lactic acid in 40 ml aliquot
dil. lactate std soln	
ml	*mg*
6.90	0.5
13.80	1.0
27.60	2.0
lactate std soln	
8.25	3.0
11.00	4.0
13.75	5.0
16.50	6.0
19.25	7.0
22.00	8.0
24.75	9.0
27.50	10.0
30.25	11.0
33.00	12.0

15.012 Extraction

Place 50 ml filtrate from prepd sample and 0.5 ml H_2SO_4 (1+1) in inner tube of extractor and connect to longest bulb-type condenser available, having outlet not $<\frac{1}{2}''$ i. d. to minimize ether regurgitation. Run H_2O thru condenser at max. condensation efficiency. Connect extn flask contg 200 ml ether, and lower flask onto preheated hot plate to prevent super-heating the ether. Protect extractor from heat of hot plate by upright sheet of asbestos and ext. all the lactic acid.

When ether in extn flask is kept at rapid boiling and condenser H_2O is cold enough to let condensed ether return to extn flask in steady stream, extn for 3 hr delivers all the lactic acid. When this rate of extn cannot be maintained because of high temp. of H_2O passing thru condenser, continue extn until equiv. of 7500 ml ether has passed thru soln. Time required, T, established for each set of new conditions, is calcd from 2 factors: A, quantity of ether necessary to fill extractor to overflowing at side-arm, which is constant for each app.; and B, time in min. required for quantity A to pass from extn flask and fill extractor.

To det. A, place 50 ml H_2O and 0.5 ml H_2SO_4 (1+1) in extractor. With extractor held upright, carefully pour ether from graduate into inner tube until it just starts passing out of side-arm. Det. B in ordinary course of starting each detn. With stop-watch, record interval from time ether first drops from condenser and falls into inner tube to time first drops return to extn flask from overflow into side-arm. Time, T, necessary for 7500 ml to pass thru app. $=7500\ B/A$. Calcd T holds true only if rate of boiling and condensing is unchanged thruout extn period.

15.013 Determination

To flask contg ether ext. add 20 ml H_2O and expel ether on steam bath. Do not let flask remain on steam bath after ether is expelled. Neutralize with *satd* $Ba(OH)_2$ *soln*, using phthln. Wash into 110 ml vol. flask with alcohol until vol. is ca 90 ml. Heat almost to boiling on steam bath, cool, dil. to mark with alcohol, and filter thru quant. paper. To expel alcohol, evap. 100 ml filtrate to ca 10 ml, add ca 50 ml H_2O, and again evap. to ca 10 ml (or evap. the 100 ml filtrate to dryness on steam bath).

Add, from buret, 6.6 ml 0.1N HCl and transfer contents of beaker with H_2O to 50–55 ml vol. flask until vol. is ca 40 ml. Add 200 mg of the prepd C, mix immediately, and keep on steam bath 10 min., mixing frequently. Cool, dil. to mark with H_2O at room temp., and filter thru quant. paper, pouring back until clear.

Transfer 40 ml filtrate to Nessler tube. (Total of 6 ml 0.1N HCl must be in tube; 40 ml filtrate contains 4.8 ml 0.1N HCl added during C treatment; to provide 6 ml 0.1N HCl required, add 1.2 ml of the acid.) Place tube in jacket of black paper, add 5 ml of the $FeCl_3$ soln from buret or pipet, dil. to 50 ml mark, and mix. (After color develops, dilg to reduce color intensity is not permissible.) Fill 4″ cell, walls painted black, with the soln, and read in photometer, using filter No. 46. (Use same cell, photometer, and color filter used in obtaining std curve. If photometer is not available, make comparisons in conventional manner with Nessler tubes.)

Det. quantity of lactic acid present in the 40 ml aliquot from std curve. If quantity of lactic acid in 40 ml portion filtrate is >12 mg limit of std curve, repeat detn on 10 ml portion of remaining filtrate. The 10 ml aliquot contains 1.2 ml 0.1N HCl, and 4.8 ml of the acid must be added to complete the 6 ml required in Nessler tube. Report lactic acid in mg/100 g.

Total Solids

15.014 *Method I.—Official*

Weigh 2.5–3 g prepd sample, **15.002**, into weighed flat-bottom dish not <5 cm diam.; use ca 5 g and Pt dish if ash is to be detd on same portion. Heat on steam bath 10–15 min., exposing max. surface of dish bottom to live steam; then heat 3 hr in air oven at 98–100°. Cool in desiccator, weigh quickly, and report % residue as total solids.

15.015 *Method II.—(Approximate)—Procedure*

Det. sp. gr. of milk with Quévenne lactometer (reading top of meniscus), observe temp., and correct reading L to 60°F by **43.024.** Calc. total solids either from formula $0.25\ L + 1.2\ F$, in which $F = \%$ fat in milk, or from **43.025.**

15.016 **Ash** (*4*)—**Official**

Into suitable Pt dish weigh ca 5 g prepd sample, **15.002,** and evap. to dryness on steam bath. Ignite in muffle at temp. not >550° until ash is C-free. Cool in desiccator, weigh, and calc. % ash.

15.017 **Total Nitrogen—Official**

Transfer 5 g sample to Kjeldahl digestion flask and proceed as in **2.036.** % N×6.38 = % "protein."

Casein

(Make detn while milk is fresh or nearly so. If delayed >24 hr, add HCHO to milk (1:2500), and keep mixt. cool.)

15.018 *Method I.—Official*

Place 10 g sample in beaker with 90 ml H_2O at 40–42° and add at once 1.5 ml HOAc (1+9). Stir and let stand 3–5 min. Decant on acid-washed filter, wash by decanting 2 or 3 times with cold H_2O, and transfer ppt to filter. Wash once or twice on filter. (Filtrate should be clear, or nearly so.) If first portions of filtrate are not clear, refilter, and complete washing ppt. Det. N in washed ppt and paper as in **2.036,** and multiply result by 6.38 to obtain equiv. of casein.

To preserved sample of milk add the dil. HOAc dropwise with stirring, and continue addn until liquid above ppt becomes clear, or very nearly so.

Method II. (*5*)—*Official*

15.019 REAGENT

Pipet 250 ml 1*N* HOAc into 1 L vol. flask. Add 125 ml CO_2-free 1*N* NaOH, dil. to vol. with CO_2-free H_2O, and mix thoroly.

15.020 DETERMINATION

Pipet 20 ml sample into 100 ml vol. flask. Add 50 ml of the reagent, mix, dil. to vol. with H_2O, and shake well. Set flask in 50–60° H_2O (*not* >60°) for 15 min. Cool to room temp., add 0.5 g Celite analytical filter-aid, shake thoroly, and filter clear thru suitable folded paper, avoiding evapn during filtration. Det. N, A, in 50 ml clear filtrate, and det. total N, B, in 10 ml of the milk. $(B - A) \times 6.38$ = casein in 10 ml of the milk. Report g casein/100 ml milk, or divide g/100 ml by density of milk and report as % by wt.

15.021 **Albumin—Official**

Exactly neutralize filtrate obtained in **15.018** with 10% NaOH soln, add 0.3 ml HOAc (1+9), and heat on steam bath until albumin is completely pptd. Collect ppt on acid-washed filter, wash with cold H_2O, and det. N as in **2.036.** N×6.38 = albumin.

Protein-Reducing Substances (*6*)—**First Action**

(Complete analyses same day they are begun. Do not permit tests to stand too long after cooling or after filtration. Oxidizing or reducing fumes (H_2S, Cl, HNO_3, etc.) must be absent from laboratory during detn.)

15.022 REAGENTS

(**a**) *Phthalate buffer soln.*—pH 5.6. Dissolve 2.0 g NaOH in H_2O and dil. to 250 ml. Dissolve 10.2 g KH phthalate in H_2O and dil. to 250 ml. Mix 159 ml NaOH soln with 200 ml phthalate soln and dil. to 800 ml in graduate. Adjust to pH 5.6 by addn of NaOH or phthalate soln.

(**b**) *Potassium ferricyanide soln.*—1%. Dissolve 10 g $K_3Fe(CN)_6$ in H_2O and dil. to 1 L. Discard if soln appears green or contains blue ppt. Prep. new std curve with each new batch of reagent.

(**c**) *Ferric chloride soln.*—0.1%. Dissolve 0.1 g or 0.1 ml liquefied portion of $FeCl_3.6H_2O$ in 100 ml H_2O. Prep. fresh daily.

15.023 APPARATUS

(**a**) *Centrifuge tubes.*—50 ml graduated, Pyrex, conical red line, Corning No. 8100.

(**b**) *Spectrophotometer.*—Beckman Model B or equiv. with matched set of 10 mm Corex cells.

15.024 PREPARATION OF STANDARD CURVE

Weigh just before use 0.1147 g $K_4Fe(CN)_6.3H_2O$ and dil. to 1 L with H_2O. Dil. 50 ml to 100 ml in vol. flask (1 ml = 0.05 mg $K_4Fe(CN)_6$). Pipet 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, and 4 ml of the dil. soln into series of clean, dry test tubes. Pipet in H_2O to give total vol. of 5.0 ml. Add to each tube 5 ml of "blank soln" prepd as follows: Dil. 3 ml *satd urea soln* to 15 ml with H_2O; add 5 ml phthalate buffer soln, 5 ml $K_3Fe(CN)_6$ soln, **15.022(b)**, and 5 ml *10% trichloroacetic acid* soln. Mix with stirring rod.

Add to each tube at convenient intervals 1 ml of the $FeCl_3$ soln, mix, and after exactly 10 min. read in spectrophotometer set to read 100% transmittance at 610 mμ with control soln (0.0 ml $K_4Fe(CN)_6$ soln). Plot % transmittance against mg $K_4Fe(CN)_6$ on semi-log paper.

15.025 DETERMINATION

Store samples at preferably ca 3°. Frozen or preserved samples are unsuitable for test.

Mix sample thoroly by pouring into container and back until homogeneous. Pipet 15 ml sample into 50 ml graduated centrifuge tube contg 15 ml H_2O. Add 3 ml 5% HOAc soln, stir thoroly, and centrifuge 5 min. at 1000–1500 rpm. Decant supernatant. (Small amount of floating curd may be disregarded; if sample contains excessive cream, curd will float and supernatant cannot be decanted. Discard test and remix sample thoroly.) Wash ppt twice with 15 ml portions H_2O, mixing ppt each time with rod, centrifuging 5 min., and decanting.

To ppt and to clean centrifuge tube as blank add 3 ml satd urea soln and then dil. to 15 ml with H_2O. Stir thoroly, add 5 ml phthalate buffer soln and 5 ml 1% $K_3Fe(CN)_6$ soln, and stir. Place in 70° H_2O bath exactly 20 min. and cool in ice-H_2O.

When cool, add 5 ml 10% trichloroacetic acid soln, stir, and filter thru 11 cm Whatman No. 40 paper, or equiv. Use first few ml filtrate to wash sides and bottom of receiver, and discard. Filter remainder of soln and let drain completely; then refilter if cloudy.

Add 5 ml H_2O to clean, dry test tube and then add 5 ml clear filtrate. Add 1 ml 0.1% $FeCl_3$ soln to develop color. Stir and let stand exactly 10 min. Read in spectrophotometer set to read 100% transmittance at 610 mμ against blank. With series of samples, add $FeCl_3$ soln at convenient intervals to permit readings 10 min. after addn. From std curve det. amount of reducing substances as mg $K_4Fe(CN)_6$ and calc. to 100 ml milk basis by multiplying by 40 (100/2.5 ml equiv. aliquot).

Lactose—Official

Polarimetric Method (7)

15.026 REAGENTS

(a) *Acid-mercuric nitrate soln.*—Dissolve Hg in twice its wt HNO_3 and dil. with 5 vols H_2O.

(b) *Mercuric iodide soln.*—Dissolve 33.2 g KI and 13.5 g $HgCl_2$ in 200 ml HOAc and 640 ml H_2O.

15.027 DETERMINATION

Weigh 65.8 g (2 normal wt) milk into each of 2 vol. flasks, 100 and 200 ml, resp. Add to each flask 20 ml of the acid-$Hg(NO_3)_2$ soln or 30 ml of the HgI_2 soln. To 100 ml flask add *5% phosphotungstic acid soln* to mark, and to the 200 ml flask add 15 ml 5% phosphotungstic acid soln and dil. to mark with H_2O. Shake both flasks frequently during 15 min., filter thru dry filter, and polarize. (It is preferable to read soln from 200 ml flask in 400 mm tube to reduce error of reading; soln from 100 ml flask may be read in 200 mm tube.) Calc. % lactose in sample as follows: (1) Subtract reading of soln from 200 ml flask (using 400 mm tube) from reading of soln from 100 ml flask (using 200 mm tube); (2) multiply difference by 2; (3) subtract result from reading of soln from 100 ml flask; (4) divide result by 2.

15.028 *Gravimetric Method*

Dil. 25 g sample with 400 ml H_2O in 500 ml vol. flask. Add 10 ml $CuSO_4$ soln, **29.035(a)**, and ca 7.5 ml KOH soln of such concn that 1 vol. is just enough to ppt completely the Cu as hydroxide from 1 vol. of the $CuSO_4$ soln. (Instead, 8.8 ml 0.5*N* NaOH may be used. After addn of alkali soln, mixt. must still be acid and contain Cu in soln.) Dil. to mark, mix, filter thru dry filter, and det. lactose in aliquot of filtrate as in **29.039.** Obtain from **43.011** wt lactose equiv. to wt Cu_2O.

Fat

15.029 *Roese-Gottlieb Method (8)—Official*

Transfer 10 g sample to Mojonnier fat-extn flask or Röhrig tube. Add 1.25 ml NH_4OH (2 ml if sample is sour) and mix thoroly. Add 10 ml alcohol and mix well. Add 25 ml ether (all ether must be peroxide-free), stopper with cork or stopper (synthetic rubber) unaffected by usual fat solvents, and shake very vigorously 1 min. Add 25 ml petr. ether (redistd slowly at temp. <65°) and repeat vigorous shaking. Centrifuge Mojonnier flask at ca 600 rpm or let it (or Röhrig tube) stand until upper liquid is practically clear. Decant ether soln into suitable flask or metal dish. Wash lip and stopper of extn flask or tube with mixt. of equal parts of the 2 solvents and add washings to weighing flask or dish. Twice repeat extn of liquid remaining in flask or tube, using 15 ml of each solvent each time.

Evap. solvents completely on hot plate or steam bath at temp. that does not cause spattering or bumping. Dry fat in oven at temp. of boiling H_2O to constant wt. Weigh cooled flask or dish, using as counterpoise duplicate container handled similarly, and avoid wiping either immediately before weighing. Remove fat completely from container with warm petr. ether, dry, and weigh as before. Loss in wt = wt fat. Correct wt fat by blank detn on reagents used.

Babcock Method (9)—Official

15.030 APPARATUS

(a) *Standard Babcock milk-test bottle.*—8%, 18 g, 6″ milk-test bottle, total height 150–165 mm (5.9–6.5″). Bottom of bottle is flat, and axis of neck is vertical when bottle stands on level surface. Charge of milk for bottle is 18 g.

(1) *Bulb.*—Capacity of bulb to junction with neck must be not <45 ml. Shape of bulb may be

either cylindrical or conical. If cylindrical, o. d. must be between 34 and 36 mm; if conical, o. d. of base must be between 31 and 33 mm, and max. diam. between 35 and 37 mm.

(2) *Neck.*—Cylindrical and of uniform diam. from at least 5 mm below lowest graduation mark to at least 5 mm above highest mark. Top of neck is flared to diam. of not <10 mm. Graduated portion of neck has length of not <63.5 mm and is graduated in whole %, 0.5%, and 0.1%, resp., from 0.0 to 8.0%. Tenths % graduations are not <3 mm long; 0.5% graduations are not <4 mm long and project 1 mm to left; and whole % graduations extend at least half-way around neck to right and project at least 2 mm to left of tenths % graduations. Each whole % graduation is numbered, with number placed to left of scale. Capacity of neck for each whole % on scale is 0.20 ml. Max. error of total graduation or any part thereof must not exceed vol. of smallest unit of graduation.

Each bottle must be constructed so as to withstand stress to which it will be subjected in centrifuge.

(3) *Testing.*—Hg and cork, alcohol and buret, and alcohol and brass plunger methods may be used for rapid testing of bottles, but accuracy of any questionable bottle must be detd by calibration with Hg (13.5471 g clean, dry Hg at 20° to be equal to 5% on scale of 18 g bottle and 10% on scale of 9 g bottle), bottle having been previously filled to zero with Hg.

(**b**) *Pipet.*—Std milk pipet conforms to following specifications:

	mm
Total length, not more than..........	330
O. d. of suction tube.................	6–8
Length of suction tube................	130
O. d. of delivery tube.................	4.5–5.5
(Must fit into bottle (**a**))	
Length of delivery tube...............	100–120
Distance of graduation mark above bulb..	15–45

Nozzle parallel with axis of pipet, but slightly constricted so as to discharge in 5–8 sec. when filled with H_2O.

Graduation, to contain 17.6 ml H_2O at 20° when bottom of meniscus coincides with mark on suction tube.

Max. error in graduation, not >0.05 ml. Pipet is to be marked "Holds 17.6 ml."

(1) *Testing.*—Test pipet by measuring from buret vol. H_2O (at 20°) which it holds up to graduation mark.

(**c**) *Acid measure.*—Device used to measure H_2SO_4, whether graduated cylinder or pipet attached to Swedish acid bottle, must be graduated to deliver 17.5 ml.

(**d**) *Centrifuge or "tester."*—Std centrifuge, however driven, must be constructed thruout and so mounted as to be capable, when filled to capacity, of rotating at necessary speed with min. vibration and without liability of causing injury or accident. It must be heated, electrically or otherwise, to temp. of at least 55° during centrifuging. It must be provided with speed indicator, permanently attached, if possible. Proper rate of rotation may be ascertained by reference to table below. By "diam. of wheel" is meant distance between inside bottoms of opposite cups measured thru center of rotation of centrifuge wheel while cups are horizontally extended.

Diam. of wheel, inches	*RPM*
14	909
16	848
18	800
20	759
22	724
24	693

(**e**) *Dividers or calipers.*—For measuring fat column.

(**f**) *Water bath for test bottles.*—Provided with thermometer and device to maintain temp. of 55–60°.

15.031 DETERMINATION

With pipet, **15.030**(**b**), transfer 18 g prepd sample, **15.002,** to milk-test bottle. Blow out milk in pipet tip ca 10 sec. after free outflow ceases. Add portionwise ca 17.5 ml H_2SO_4 (sp. gr. 1.82–1.83 at 20°) tempered at 15–20°, washing all traces of milk into bulb. Shake until all traces of curd disappear; place bottle in heated centrifuge, counterbalance, and after proper speed is reached, whirl 5 min. Add soft H_2O at 60°, or above, until bulb of bottle is filled. Whirl 2 min. Add hot H_2O until liquid column approaches top graduation of scale. Whirl 1 min. longer at 55–60°. Transfer bottle to warm H_2O bath kept at 55–60°, immerse it to level of top of fat column, and leave until column is in equilibrium and lower fat surface assumes final form (not <3 min.). Remove bottle from bath, wipe it, and with aid of dividers or calipers measure fat column, in terms of % by wt, from lower surface to highest point of upper meniscus.

Fat column, at time of measurement, should be translucent, golden-yellow or amber, and free from visible suspended particles. Reject all tests in which fat column is milky or shows presence of curd or of charred matter, or in which reading is indistinct or uncertain; repeat test, adjusting quantity of H_2SO_4 added.

Rapid Detergent Method for Raw Milk (10)—
First Action

15.032 REAGENTS

(**a**) *Solid detergent reagent.*—Grind together 3 parts (by wt) urea, 3 parts Na_2CO_3, 2 parts ethylenediaminetetraacetic acid (EDTA), and 1

part Na_2HPO_4 until finely divided and lump-free. Add 4 parts polyoxyethylene esters of mixed fatty and resin acids (16 EtO/mole) (available under designation "PFR-16" from Atlas Powder Co., Commercial Division, Wilmington, Del. or from Technical Industries, 2711 S.W. Second Ave., Fort Lauderdale, Fla.) and mix thoroly by mixing and rubbing thru No. 8 sieve until mass is lump-free and has soft, moldable consistency. Store in dry place at room temp. at least 10 days to react and set up. Break up dry, hard cake, reduce to free-flowing powder, pass thru No. 20 sieve, and mix thoroly. (If cake cannot be powdered, let it age longer.) (Prepd reagent is available as "TeSa Reagent Concentrate ®" from Technical Industries.)

(**b**) *Fat test reagent.*—Dissolve 156 g solid reagent, (**a**), in distd or good quality (drinking) tap H_2O and dil. to 1 L. Mix thoroly and let stand at least 6 hr before use. Prep. fresh every 2 weeks.

(**c**) *Milk test reagent.*—Mix 1 vol. MeOH with 5 vols fat test reagent, (**b**), and mix thoroly. Prep. fresh every 2 days.

15.033 APPARATUS

(**a**) *Milk test bottles with side neck.*—8%, 18 g, total height 160–175 mm. Bottom of bottle is flat, and axis of neck is vertical when bottle stands on level surface. Charge of milk for bottle is 18 g. (Available as "TeSa Milk Test Bottles ®" from Technical Industries, **15.032**(**a**).)

(1) *Bulb.*—Capacity of bulb to junction with center neck must be not <48 ml. Shape of bulb is cylindrical with max. o.d. not >37 mm. Min. taper of bulb to junction with center neck at any point must be not >50° to axis of center neck.

(2) *Center neck.*—Cylindrical and of uniform diam. from at least 5 mm below lowest graduation mark to 5 mm above highest mark. Top of neck is flared to diam. not <10 mm and highest graduation mark must be 17–20 mm below open, flared top. Graduated portion of neck has length not <65 mm and is graduated in whole %, 0.5%, and 0.1%, resp., from 0.0 at top downward to 8%. Tenths % graduations are not <3 mm long; 0.5% graduations are not <4 mm long and project 1 mm to left; and whole % graduations extend at least halfway around neck to right and project at least 2 mm to left of tenths % graduations. Each whole % graduation is numbered, with number placed to left of scale. Capacity of neck for each whole % on scale is 0.2 ml. Max. error of total graduation or any part thereof must not exceed one-half vol. of smallest unit graduation.

(3) *Secondary side arm tube.*—Tube, 7–8 mm o.d., is sealed into tapered portion of bulb at ca right angle to taper so that closest portion of seal to junction of bulb and center neck is not <5 mm. Portion of tube within bulb is bent and extends downward parallel to axis of center neck, and terminates in open end 2–6 mm from inside bottom of bulb. Portion of tube outside bulb is bent and extends upward parallel to axis of center neck at distance 2–5 mm from neck and terminates in open end flared to diam. not <10 mm at 2–5 mm above highest graduation of center neck. Tube is placed opposite center neck so that it does not obstruct reading of scale. Each bottle is constructed to withstand stresses required in normal use.

(4) *Testing.*—*See* **15.030**(**a**)(3).

(**b**) *Pipet.*—*See* **15.030**(**b**).

(**c**) *Reagent measure.*—Any device graduated to deliver 15 ml.

(**d**) *Water baths.*—(1) *Reaction bath.*—Provided with thermometer and heater for maintaining H_2O at b.p. (97–100°). If altitude is such that b. p. is <97°, add glycerine or ethylene glycol to raise b.p. to not >100°. (2) *Tempering bath.*—*See* **15.030**(**f**).

15.034 DETERMINATION

With pipet, **15.030**(**b**), transfer 18 g prepd sample, **15.002**, to milk test bottle, **15.033**(**a**), thru side tube. Blow out milk in pipet tip ca 10 sec. after free outflow ceases. Add 15 ml milk test reagent, **15.032**(**c**), thru side tube, washing all traces of milk into bulb, and immediately swirl to obtain uniform mixt. Place bottle in boiling H_2O bath 10–12 min. Remove bottle and slowly add hot H_2O thru side arm until full. Let stand at room temp. 5–6 min. Place bottle in tempering bath to level of top graduation 3 min. Add H_2O at 55–60° thru side arm to raise fat level to, or just below, 0 graduation. Read lower meniscus and report difference between upper and lower menisci in % by wt of fat. (Upper meniscus may also be adjusted to exact 0 mark by inserting small rod into side tube, or fat column may be measured with calipers.) (Read meniscus as in usual volumetric measurements—not as in Babcock tests.)

If small amount of foam or undigested material obscures lower meniscus, add few drops 40% MeOH thru fat column, retemper, and read as above.

If large amount of undigested material obscures lower meniscus, repeat test, using 17–18 ml milk test reagent, and leaving in boiling H_2O bath 15–20 min., shaking 3 or 4 times during first 10 min.

Added Water

15.035 *Acetic Serum Method* (*11*)
Official

(**a**) *Zeiss immersion refractometer reading.*—To 100 ml sample, measured at 20° into beaker, add

2 ml 25% HOAc (sp. gr. 1.035). Cover beaker with watch glass, keep in H_2O bath 20 min. at 70°, then in ice-H_2O 10 min., and sep. curd from serum by rapid filtration thru small filter. Transfer portion of clear serum to refractometer beaker, place in constant temp. bath, and take refractometer reading when temp. of serum is exactly 20°, as detd by thermometer graduated in 0.1°. (Scale readings are identical on Bausch and Lomb refractometers except those with serial Nos. 4000–10,000, for which readings of 38.6 and 39.6 correspond, resp., to 39 and 40 on Zeiss instrument. (Zeiss scale = B & L scale × 1.0092.))

(b) *Ash.*—Transfer 25 ml serum to weighed flat-bottom Pt dish and evap. to dryness on H_2O bath. Heat over low flame (to avoid spattering) until contents are thoroly charred, place dish in muffle, preferably with pyrometer control, and ignite to white ash at temp. not >500°. Cool and weigh. Express result as g/100 ml.

15.036 *Copper Serum Method (12)—Official*

To 1 vol. $CuSO_4$ soln (72.5 g $CuSO_4.5H_2O$/L, adjusted if necessary to read 36 at 20° on scale of Zeiss immersion refractometer, or to sp. gr. of 1.0443 at 20/4°), add 4 vols milk. Shake well and filter. Det. refractometer reading of clear serum at 20°. (Scale readings are identical on Bausch and Lomb immersion refractometers except those with serial Nos. 4000–10,000 for which reading of 35.6 corresponds to 36 on Zeiss instrument.)

Cryoscopic Method (13)—Official

15.037 APPARATUS

(a) *Cryoscope.*—*See* Fig. 27. 1 L cylindrical Dewar flask, 28 cm internal depth, encased in metal, is tightly closed by large cork ca 3 cm thick. Thru center of cork is tightly fitted, medium thin wall glass or metal tube, 250 mm long × 33 mm o. d. At one side of cork is inserted narrow metal inlet tube, lower end of which is formed into perforated loop near bottom of flask. At opposite side is T-shaped metal tube 6 mm i.d., for escape of vapors, and also for addn of volatile fluid into app. At back portion of cork is fitted control thermometer, bulb of which extends nearly to bottom of flask.

Freezing test tube is of thin glass, ca 240 mm long × 29 mm o.d., and fits closely into larger tube sealed into cork. In rubber stopper of freezing tube is fitted the std thermometer so that bottom of thermometer is 15 mm above bottom of sample tube. Length of thermometer permits insertion of bulb nearly to bottom of tube and at same time allows complete exposure of scale above stopper. At right side of thermometer stirring device made of non-corrodible low conductivity

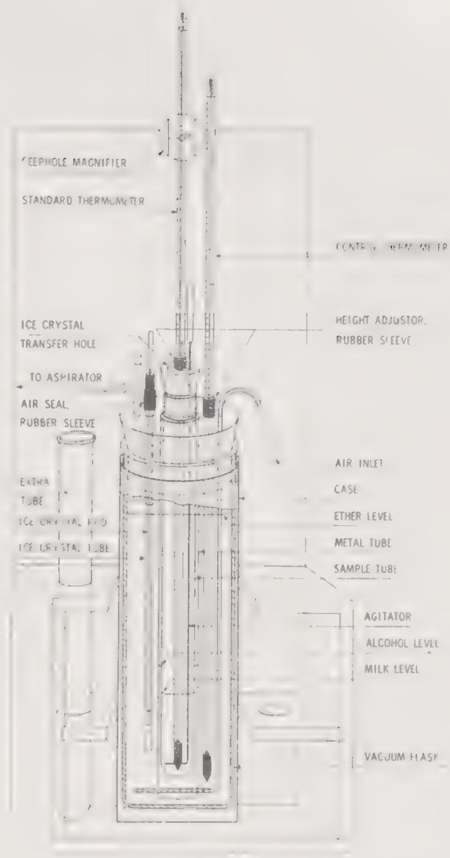

FIG. 27.—HORTVET CRYOSCOPE

metal is fitted into stopper thru short section of thin-wall metal tubing; lower end extends nearly to bottom of test tube and is provided with horizontal loop encircling thermometer. At left of thermometer is freezing-starter attachment inserted thru opening in stopper formed by short section of metal tubing. This device consists of non-corrodible metal rod, at lower end of which is opening 10 mm long to carry small fragment of ice.

At one side of cryoscope is installed air-drying arrangement which consists of Folin absorption bulb inserted thru tightly fitting stopper and extending nearly to bottom of large test tube. Short section of glass tubing is inserted thru second opening in stopper and is connected to vaporizing tube which enters cryoscope. H_2SO_4 is poured into drying tube to level slightly above small inner bulb.

At opposite side of app. is arranged a drain tube to conduct vapors away from operator. Pressure or suction pump forces dry air into app. at suitable rate and conducts mixed vapors out thru base of drain tube into sink.

Adjustable lens is mounted in convenient position in front of thermometer to magnify the scale. *See* Fig. 27.

(**b**) *Standard thermometer.*—Solid-stem instrument 58 cm total length, with scale portion measuring ca 30 cm. Total scale range is 3°, from +1° to −2°, and each degree division is subdivided into tenths and hundredths. Length of 1° division is ca 10 cm, thus making smallest subdivisions of such magnitudes as to enable easy observation and readings estimated to 0.001°. Stdze thermometer as in **15.038.** Check f.p. of freshly distd H_2O at beginning of each day. If f.p. deviates >0.002° from that obtained on H_2O when thermometer was stdzd, check to det. if Hg column is sepd. (Rejoin column as in (**d**).) If Hg column is not sepd, restdze thermometer.

(**c**) *Control thermometer.*—Solid-stem instrument ca 58 cm long with scale range of +20° to −30°. Test in bath of melting crushed ice to det. whether 0-mark on scale is correct. Scale graduations should be accurate to within 0.10°.

(**d**) *Care of Hortvet thermometers.*—Store thermometers vertically in refrigerator. Do not wash in warm H_2O. Examine before use to det. that Hg column is not sepd. If sepd, rejoin by tapping thermometer gently. If necessary warm thermometer slightly but avoid excessive warming which may alter the zero.

15.038 STANDARDIZATION OF THERMOMETER

Make 3 f.p. detns as in **15.039** on each of following:

(**a**) *Recently boiled distilled water.*

(**b**) *Sucrose soln.*—Dissolve 7 g pure sucrose in H_2O and dil. to 100 ml at 20°.

(**c**) *Sucrose soln.*—Dissolve 10 g pure sucrose in H_2O and dil. to 100 ml at 20°.

(Pure sucrose may be obtained from National Bureau of Standards, Washington 25, D. C.)

Tabulate results in following form:

FREEZING-POINT OBSERVATIONS	PURE WATER	7 GRAMS SUCROSE SOLUTION		10 GRAMS SUCROSE SOLUTION	
		Observed freezing point (−S)	Freezing-point depression S−W (algebraic)	Observed freezing point (−S)	Freezing-point depression S−W (algebraic)
1st					
2nd					
3rd					
Averages	±*W*	xxxxxxx		xxxxxxx	

Express results as degrees freezing-point depression below av. of observed f.ps. obtained on sample of pure H_2O (±W), which may be above (+) or below (−) 0-mark on scale. Obtain each f.p. depression of the sucrose solns by algebraically subtracting average of f.p. readings of pure H_2O (±W) from each observed f.p.

Omit adventitious results, *i.e.*, results that are in marked disagreement with other results obtained by carefully following instructions.

Apply av. of f.p. depressions obtained on std sucrose solns for correcting thermometer readings obtained on sample of milk in manner illustrated in tables accompanying Fig. 28.

15.039 DETERMINATION

(If titratable acidity, **15.004**, is >0.18%, results may underestimate actual amount of added water in sample.)

Insert funnel-tube into vertical portion of T-tube at one side of app. and pour in 400 ml ether previously cooled to 10° or lower. Close vertical tube with small cork and connect pressure pump to inlet tube of air-drying attachment. Adjust pump to pass air thru app. at moderate rate, judged by agitation of H_2SO_4 in drying tube. Continuous vaporization of ether causes lowering of temp. in flask from room temp. to 0° in 5–10 min. Continue temp. lowering until control thermometer registers near −3°. At this stage, by lowering gauge tube into ether bath, then closing top with forefinger and raising to suitable height, estimate can be made of quantity of ether necessary to pour in to restore the 400 ml vol. After adjusting vol. ether to 400 ml, successive addns of 10–15 ml for each sample are usually sufficient.

Pour into freezing test tube enough H_2O (30–35 ml), boiled and cooled to 10° or lower, to submerge thermometer bulb. Insert thermometer together with stirrer and lower test tube into larger tube. Small quantity of alcohol, enough to fill lower space between 2 test tubes, serves to complete conduction medium between freezing bath and liquid to be tested. Keep stirrer in steady up-and-down motion of ca 1 stroke every 1 or 2 sec., or even at slower rate, provided cooling proceeds satisfactorily.

Keep air passing thru app. until temp. of cooling-bath reaches −2.5° (top of Hg thread in thermometer usually recedes to position near f.p. of H_2O). Keep temp. of cooling bath at −2.5° and continue manipulating stirrer until super-cooling of sample of 1.0–1.2° is observed. As a rule, at this time liquid begins to freeze, indicated by rapid rise of the Hg. Manipulate stirrer slowly and

carefully 3 or 4 times as Hg column approaches its highest point, *i.e.*, ca 0.07° below expected f.p. With suitable light-wt mallet tap top of thermometer cautiously and continuously until top of Hg column remains stationary at least 1 min. Avoiding parallax, observe exact reading on scale and estimate to 0.001°. When observation is satisfactorily completed, make duplicate detn; then remove thermometer and stirrer, and empty H_2O from freezing tube.

Rinse tube with ca 25 ml sample of milk, cooled to 10° or lower; measure 30–35 ml milk into tube or enough to submerge thermometer bulb, and insert tube into app. Keep temp. of cooling-bath at 2.5° below probable f.p. of sample. Make detn on the milk as performed in detg f.p. of H_2O. As a rule, however, it is necessary to start freezing action in milk by inserting freezing-starter (kept in contact with ice for several min., and in open end of which is wedged fragment of ice) at time when Hg column has receded to 1.0–1.2° below probable f.p. Rapid rise of the Hg results almost immediately.

Remove starter and manipulate stirrer slowly and carefully 2 or 3 times when Hg approaches its highest point. Complete adjustment of Hg column in same manner as in preceding detn; then, avoiding parallax, observe exact reading on thermometer scale and estimate to 0.001°. Make duplicate detn on new aliquot of milk. *Algebraic difference* between averages of readings obtained on the H_2O and readings obtained on sample of milk represents *f.p. depression* of the milk. To det. true *f.p.* (T') of milk, subtract from f.p. depression, f.p. depression of 7% sucrose soln as detd by laboratory thermometer. Multiply difference by correction factor for thermometer. Add to product 0.422 (f.p. depression of 7% sucrose soln by NBS thermometer). See example in Fig. 28.

Presence of added H_2O is indicated if f.p. of milk is above −0.530°. It should not be assumed that milk with f.p. below −0.530° is necessarily

Laboratory Thermometer No. 2.

WATER	7 GRAMS SUCROSE TO 100 ML	10 GRAMS SUCROSE TO 100 ML
Av. +0.056°	−0.425°	−0.621°

Interval = 0.196
0.196 equiv. 0.199
Correction factor = 1.015

Laboratory Thermometer No. 24.

WATER	7 GRAMS SUCROSE TO 100 ML	10 GRAMS SUCROSE TO 100 ML
Av. 0.000°	−0.420°	−0.625°

Interval = 0.205
0.205 equiv. 0.199
Correction factor = 0.971

Example:

Laboratory Thermometer No. 24.
F. p. Depression Sample Milk = 0.548
(0.548 − .420) 0.971 = 0.124
True f. p. = 0.422 + 0.124
(= 0.546° below zero C)

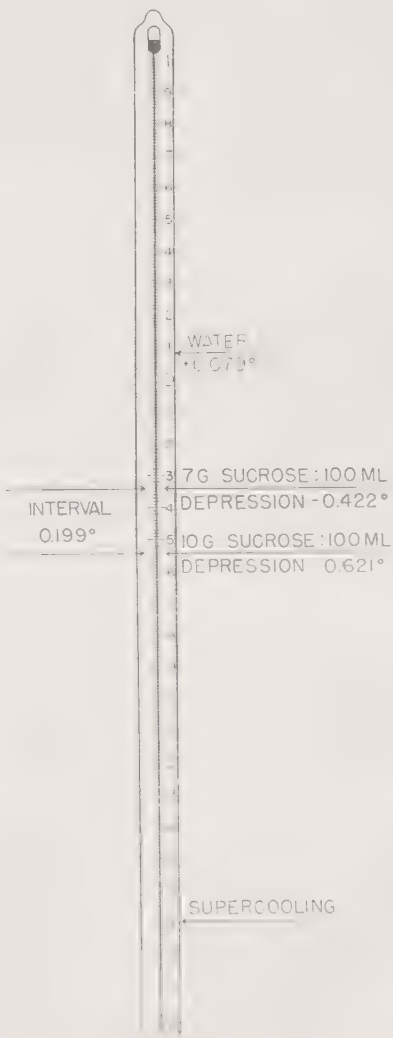

FIG. 28.—NATIONAL BUREAU OF STANDARDS TESTED THERMOMETER

free of added H_2O. In fact, samples representing large mixed lots of milk will probably have f.p. below $-0.540°$. Such milk with f.p. above $-0.540°$ should be regarded with suspicion as well as large fluctuations in f.p of bulk milk from day to day. If desired "min. % added H_2O," W, can be calcd as $W=(100-TS)(T-T')/T$, where $T=-0.530$, T'=f.p. of sample, and TS = % total solids.

Gelatin (*14*)

15.040 *Qualitative Test—Official*

To 10 ml sample add 10 ml *acid-$Hg(NO_3)_2$ soln* (Hg dissolved in twice its wt HNO_3 and this soln dild to 25 times its vol. with H_2O). Shake mixt., add 20 ml H_2O, shake again, let stand 5 min., and filter. If much gelatin is present, filtrate will be opalescent and cannot be obtained quite clear. To portion of filtrate in test tube add equal vol. *satd aq. picric acid soln.* Yellow ppt is produced in presence of any considerable quantity of gelatin; smaller quantities are indicated by cloudiness.

NOTE: In applying this test to sour, fermented, cultured, or very old samples of milk, cream, or buttermilk; to sterilized cream or evaporated milk; or to cottage cheese, use care to recognize ppts produced by picric acid when added to the $Hg(NO_3)_2$ filtrates from these materials in absence of gelatin. Such samples, with or without rennet and entirely free from gelatin, give, on standing, distinct ppts when treated as above outlined. In every case, however, these ppts differ in character from those produced by picric acid with gelatin.

Gelatin-picric acid ppt is finely divided, more apt to remain in suspension, settles only slowly, and adheres tenaciously to sides and bottom of container, from which it is rinsed with difficulty. Ppts produced by picric acid in absence of gelatin are flocculent, sep. readily (leaving serum practically clear), do not adhere to walls of container, and are easily removed by rinsing with H_2O. When gelatin is present in sample, gelatin-picric acid ppt will remain in suspension long after the flocculent ppt has settled, but on standing overnight the characteristic sticky deposit will be found adhering tenaciously to bottom and sides of test vessel. If gelatin is present in relatively high concn (1%), the gelatin-picric acid ppt will be voluminous and will settle rather quickly.

15.041 **Preservatives—Official**

Proceed as in Chap. **27**. To test for benzoic acid or salicylic acid, acidify 100 ml milk with 5 ml HCl (1+3), shake until curdled, filter, and treat clear filtrate as in **27.006**, and **27.073** or **27.074**.

To test for HCHO proceed as in **27.031–27.033**.

Hypochlorites and Chloramines (*15*)—Procedure

(Unreliable in presence of >2.5 ppm Cu)

15.042 REAGENTS

(**a**) *Potassium iodide soln.*—Dissolve 7 g KI in 100 ml H_2O. Prep. fresh.

(**b**) *Dilute hydrochloric acid.*—To 100 ml HCl add 200 ml H_2O.

(**c**) *Starch soln.*—Boil 1 g starch in 100 ml H_2O. Cool before using.

15.043 TESTS

(**a**) To 5 ml milk in test tube add 1.5 ml of the KI soln, mix thoroly by shaking, and note color of milk.

(**b**) If unaltered, add 4 ml of the HCl, mix thoroly with flat-end stirring rod, and note color of curd.

(**c**) Next place tubes in large H_2O bath, previously heated to 85°, and let stand 10 min. (during this interval curd rises to surface); then cool rapidly by placing in cold H_2O. Note color of curd and liquid.

(**d**) Then add 0.5–1 ml of the starch soln to liquid below curd and note color.

15.044 *Reactions with the various tests*

CONCENTRATION OF AVAILABLE CL	1:1,000	1:2,000	1:5,000	1:10,000	1:25,000	1:50,000
Test **a**	Yellowish brown	Deep yellow	Pale yellow, fades	—	—	—
Test **b**	Yellowish brown	Deep yellow	Light yellow	—	—	—
Test **c**	Yellowish brown	Deep yellow	Yellow	Yellow	Pale yellow	Yellowish
Test **d**	Blue purple	Blue purple	Blue purple	Dark red-purple	Red purple	Pale red-purple

15.045 **Coloring Matters** (*16*)**—Official**

Warm ca 150 ml milk in casserole over flame, add ca 5 ml HOAc (1+3), and continue to heat slowly nearly to b.p. while stirring. Gather curd, when possible, into one mass with stirring rod and pour off whey. If curd breaks up into small flecks, sep. from whey by straining thru sieve or colander. Press curd free from adhering liquid, transfer to small flask, macerate with ca 50 ml ether, keeping flask tightly corked and shaking at intervals, and

let stand several hr, preferably overnight. Decant ether ext. into evapg dish, remove ether by evapn, and test fatty residue for annatto as in **35.015**(**b**).

Curd of uncolored milk and milk colored with annatto is perfectly white after complete extn with ether. If extd fat-free curd is distinctly orange or yellowish, coal-tar dye is indicated. In many cases if lump of fat-free curd in test tube is treated with little HCl, color changes to pink, indicating presence of dye similar to aniline yellow or butter yellow or perhaps one of the acid azo yellows or oranges. In such cases, sep. and identify coloring matter present in curd as in Chap. **35.**

In some cases presence of coal-tar dyes can be detected by treating ca 100 ml milk directly with equal vol. HCl in porcelain casserole, giving dish slight rotary motion. In presence of some dyes sepd curd becomes pink.

15.046 Sediment Test—Official—*See* **36.009–36.012**

Residual Phosphatase

Method I.—Official

15.047 COLLECTION OF SAMPLE

Proceed as in **15.001,** except to be assured that no preservative is present and not >48 hr has elapsed between time of sampling and receipt at laboratory. If samples are refrigerated, avoid freezing.

15.048 REAGENTS

(**a**) *Buffer substrate.*—Dissolve 1.09 g disodium phenyl phosphate and 11.54 g Na diethyl barbiturate in H_2O satd with $CHCl_3$ and dil. to 1 L. Add 10 ml $CHCl_3$/L and store reagent in refrigerator.

(**b**) *Folin-Ciocalteu phenol reagent.*—Dissolve 100 g $Na_2WO_4.2H_2O$ (according to Folin) and 25 g $Na_2MoO_4.2H_2O$ in 700 ml H_2O in 1500 ml flask connected by $ joint to reflux condenser. Add 50 ml H_3PO_4 and 100 ml HCl, and reflux gently 10 hr. Cool, and add 150 g Li_2SO_4, 50 ml H_2O, and 4–6 drops liquid Br. Boil mixt. without condenser 15 min. to remove excess Br. Cool, transfer to 1 L flask, dil. to vol. with H_2O, and filter. (Finished reagent should be golden yellow; reject if it has greenish tint.) Keep reagent in refrigerator, protected from dust. For use dil. 1 vol. of this stock reagent with 2 vols H_2O.

(**c**) *Sodium carbonate soln.*—Prep. 14% or 1.32*M* soln of anhyd. Na_2CO_3.

(**d**) *Filter paper.*—Free from phenol or other interfering substances (Whatman No. 40 and Eaton-Dikeman "New Filt," Nos. 1 and 3, have been found satisfactory).

15.049 PERMANENT PHENOL STANDARDS

(**a**) *Color soln, gray.*—Dissolve 31.9 g $CoCl_2.6H_2O$, 67.5 g $CuSO_4.5H_2O$, and 75 g $NiSO_4.6H_2O$ in H_2O. Add 32 ml HCl and 45 ml H_2SO_4, and dil. to 500 ml.

(**b**) *Color soln, red.*—Dissolve 476 g $CoCl_2.6H_2O$ in H_2O and filter. To filtrate add 100 ml HCl and dil. to 1 L.

(**c**) *Color soln, blue.*—Dissolve 300 g $CuSO_4.5H_2O$ in H_2O, add 20 ml H_2SO_4, and dil. to 1 L. (Should crystals appear when soln is cooled below 20°, warm slightly before using to insure complete solubility.)

Prep. permanent color stds equiv. to phenol concns of 0.01–0.15 mg/0.5 ml sample by combining quantities of color solns, **a, b,** and **c** indicated in **15.050** and dilg to 10 ml with H_2O in each case; *e.g.,* 0.3 ml Soln (**a**) +0.106 ml Soln (**b**) +0.96 ml Soln (**c**) $+H_2O$ to make 10 ml is equiv. to phenol concn of 0.01 mg in 0.5 ml sample.

These color stds are intended for use only in natural daylight. If, however, turquoise blue, unglazed, opaque glass plate is used to deflect light from daylight lamp thru tubes of stds and sample, accurate color comparisons can be made in absence of daylight. Use 13 mm i.d. tubes for both stds and unknowns.

15.050 *Preparation of permanent phenol standards*

PHENOL	COLOR SOLUTION		
	GRAY (a)	RED (b)	BLUE (c)
mg/0.5 ml	*ml*	*ml*	*ml*
0.01	0.30	0.106	0.96
0.02	0.40	0.140	1.16
0.03	0.55	0.180	1.65
0.04	0.65	0.216	2.10
0.06	0.92	0.286	3.00
0.09	1.30	0.326	4.40
0.12	1.70	0.360	5.70
0.15	2.50	0.396	7.10

15.051 PREPARATION OF STANDARD CURVE FOR USE WITH PHOTOMETER

Using stock phenol soln, **15.054**(**g**)(**1**), prep. dilns so that 0.5 ml of each contains 0.01, 0.02. 0.03, 0.04, 0.06, 0.09, 0.12, and 0.15 mg phenol. For each std take 10 ml buffer substrate soln, add 4.5 ml of the dild Folin-Ciocalteu reagent and 0.5 ml of the std dil. phenol soln (or H_2O for blank), mix thoroly, let stand 3 min., and filter. To 5 ml filtrate add 1 ml of the Na_2CO_3 soln, mix thoroly by rotating tube, heat in boiling H_2O bath 5 min., and filter. Cool, and read color in photometer, using 650 mμ filter. Plot std curve of photometer readings against mg phenol without correcting for blank.

15.052 DETERMINATION

Transfer 10 ml buffer substrate soln into test tube 20×160 mm and add 0.5 ml sample. Add few drops $CHCl_3$, mix thoroly by rotating tube, and cover to protect from dust. (Do not use rubber or cork stoppers; paper toweling placed over open end of tube is satisfactory.) Warm to 37–39° in H_2O bath and incubate at 34–37° not <18 and not >24 hr. Add 4.5 ml of the dild Folin-Ciocalteu reagent, mix, and let stand 3 min. Filter, and transfer 5 ml filtrate to 13 mm i. d. test tube. Add 1 ml of the Na_2CO_3 soln and mix thoroly by rotating tube. Place tube in boiling H_2O bath 5 min. and filter. Cool, and estimate color of filtrate by comparison with permanent color stds in 13 mm i.d. tube, or with photometer and 650 mμ filter.

15.053 CONTROL TEST

(To check deterioration of reagents and/or presence of interfering substances in sample)

To 10 ml of the buffer substrate soln add 4.5 ml of the dild Folin-Ciocalteu reagent and 0.5 ml sample. (Do not incubate.) Mix thoroly, let stand 3 min., and filter. To 5 ml filtrate add 1 ml of the Na_2CO_3 soln, mix thoroly by rotating tube, heat in boiling H_2O bath 5 min., and filter. Cool, and compare color of filtrate with permanent color stds in 13 mm i. d. tube, or with photometer and 650 mμ filter. If phenol value obtained is >0.02 mg, subtract excess from phenol value of incubated sample to obtain phenol value indicative of pasteurization treatment.

Phenol value of 0.047 mg phenol/0.5 ml sample generally indicates milk heated at 143°F 30 min. Value greater than this indicates progressively inadequate heat treatment. In reporting results, give mg phenol/0.5 ml sample as well as interpretation as to whether the milk is pasteurized or underpasteurized.

Method II(17)—Official*

15.054 REAGENTS

(a) *Buffers:*

(1) *Barium borate-hydroxide buffer.*—pH 10.6 ±0.15 at 25°. Dissolve 25.0 g $Ba(OH)_2.8H_2O$ (fresh, not deteriorated) in H_2O and dil. to 500 ml. Separately dissolve 11.0 g H_3BO_3 and dil. to 500 ml. Warm each soln to 50°, mix solns, stir, cool to ca 20°, filter, and keep filtrate in tightly stoppered container. (For use with milk dil. 500 ml of this buffer with 500 ml H_2O.)

(2) *Color development buffer.*—pH 9.8±0.15 at 25°. Dissolve 6.0 g Na metaborate ($NaBO_2$) and 20 g NaCl in H_2O, and dil. to 1 L with H_2O.

(3) *Color dilution buffer.*—Dil. 100 ml color development buffer, (2), to 1 L with H_2O.

(4) *Borax std buffer for checking pH meter.*—0.01*M*, pH 9.18 at 25°, **42.007**(e).

(b) *Buffer substrates:*

(1) *For evaluating pasteurization.*—Dissolve 0.10 g phenol-free cryst. disodium phenyl phosphate in 100 ml mixt. of the Ba borate-hydroxide buffer, (a)(1), with equal vol. H_2O. (Cryst. $Na_2C_6H_5PO_4$ should be stored in freezing compartment of refrigerator or in desiccator.) If the $Na_2C_6H_5PO_4$ is not phenol-free, purify it as follows: Dissolve 0.5 g in 4.5 ml H_2O, add 0.5 ml buffer (a)(1) and 2 drops of the BQC reagent, (d), and let stand 30 min. Ext. color with 2.5 ml butyl alcohol, (f), and let stand until alcohol seps. Remove alcohol with dropper and discard. Dil. 1.0 ml aq. soln to 100 ml with dil. Ba borate-hydroxide buffer, (a)(1), for prepn of buffer substrate. This stock soln may be stored in refrigerator few days, but develop color and re-ext. before use.

(2) *For quantitative results with raw milk.*—Prep. as in (1), except to use 0.20 g $Na_2C_6H_5PO_4$ or 2.0 ml of the purified soln.

(c) *Zinc-copper protein precipitant.*—Dissolve 3.0 g $ZnSO_4.7H_2O$ and 0.6 g $CuSO_4.5H_2O$ in H_2O and dil. to 100 ml with H_2O.

(d) *BQC (2,6-dibromoquinonechloroimide) soln (Gibbs reagent).*—Dissolve 40 mg BQC powder in 10 ml absolute alcohol or MeOH and transfer to dark-color dropper bottle. (Reagent remains stable at least 1 month if kept in ice tray of refrigerator; do not use after it begins to turn brown. Store powd. BQC in freezing compartment of refrigerator or in desiccator. Check new lots of BQC before use by prepg std curve with phenol and comparing curve obtained with that from lot of BQC known to be suitable. Repeat this procedure at least semi-annually.)

(e) *Copper sulfate soln for standards.*—0.05%. Dissolve 0.05 g $CuSO_4.5H_2O$ in H_2O and dil. to 100 ml.

(f) *Butyl alcohol.*—Use *n*-butyl alcohol, b.p. 116–118°. To adjust pH, mix 1 L with 50 ml color development buffer, (a)(2). Store in g-s. container.

(g) *Phenol stds:*

(1) *Stock soln.*—Weigh accurately 1.000 g pure phenol, transfer to 1 L vol. flask, dil. to mark with H_2O, and mix (1 ml =1 mg phenol). (This stock soln remains stable several months in refrigerator.)

(2) *Standards.*—Dil. 10.0 ml of the stock soln, (1), to 1 L with H_2O and mix (1 ml=10 mmg, 0.00001 g, or 10 units of phenol). Use this std soln to prep. more dil. std solns: *e.g.*, dil. 5, 10, 30, and 50 ml to 100 ml with H_2O to prep. std solns contg 0.5, 1.0, 3.0, and 5.0 mmg or units of phenol/ml.

* All glassware, stoppers, and sampling tools must be scrupulously clean and [illegible] desirable to [illegible] them in hot running H_2O after cleaning. Phenolic plastic closures on reagent bottles may cause phenolic contamination and their use should be avoided.

resp. Keep these std solns in refrigerator not >1 week.

In similar manner prep. from the stock soln std solns contg 20, 30, and 40 units/ml.

Measure appropriate quantities of std solns into series of tubes (preferably graduated at 5.0 and 10.0 ml) to provide suitable range of stds as needed, contg 0 (control or blank), 0.5, 1.0, 3.0, 5.0, 10.0, 20.0, 30.0, and 40.0 units. To increase brightness of blue color and improve stability of std, add to each tube 1.0 ml $CuSO_4$ soln, (**e**). Then add 5.0 ml color diln buffer, (**a**)(3), and dil. to vol. of 10.0 ml with H_2O. Add 4 drops (0.08 ml) BQC soln, (**d**), mix, and let blue color develop 30 min. at room temp. If butyl alcohol extn method is used, ext. stds as in **15.056**, *Step 10*(*b*).

Read color intensities in photometer with 610 mμ filter, subtract value of blank from value of each phenol std, and prep. std curve (should be straight line).

If stds are to be used for visual comparison, store in refrigerator. Prep. new set weekly.

15.055 SAMPLING

Mix product well, pour several ml into small tube, stopper, and keep in refrigerator. If preservative is necessary, add 1–3% $CHCl_3$, and label "*Poison, preservative added.*"

15.056 DETERMINATION

Chemical principles involved in detection and measurement of milk phosphatase activity are same for all dairy products, but different dairy products require modifications of methods because of their different physical properties, compositions, and especially buffering capacities.

For milk and other fluid products, proceed as follows:

Step 1.—Pipet 1.0 ml portions of sample into 2 or 3 tubes (one tube is needed for control or blank; it is preferable to have 2 more tubes for duplicate detns). (For goat's milk, use 3 ml portions.)

Step 2.—Heat *blank* ca 1 min. in covered beaker of boiling H_2O (temp. of entire tube must be 85–90°) and cool to room temp. From this point on, treat blank and test identically.

Step 3.—Add 10.0 ml Ba buffer substrate, **15.054**(**b**)(1) or (2), stopper tube, and mix (pH 10.0±0.15).

(This substrate is satisfactory for fresh milk, sweet buttermilk, or cheese whey. For old or slightly sour milk use substrate prepd from undild buffer, **15.054**(**a**)(1); for chocolate drinks prep. substrate from buffer dild with ¼ vol. H_2O; for very acid (pH <4.5) buttermilk prep. substrate from the 26-11 buffer, **15.160**(**a**)(2); and for goat's milk prep. substrate from the 27-11 buffer, **15.160**(**a**)(2).

For precise quant. results on unknown samples, adjust pH to 10.0–10.05.

Step 4.—Immediately after adding substrate, incubate in H_2O bath 1 hr at 37–38°, mixing or shaking contents occasionally.

Step 5.—Heat in beaker of boiling H_2O nearly 1 min. (Temp. of contents of tubes should reach 85–90°, as detd by thermometer in another tube of same size and shape contg same vol. liquid.) Cool to room temp. in vessel of cold H_2O.

Step 6.—Pipet in 1.0 ml Zn-Cu protein precipitant, **15.054**(**c**), for fresh milk, sweet buttermilk, or cheese whey. (For old or slightly sour milk or acid buttermilk substitute 1.0 ml 6.0 g/100 ml $ZnSO_4.7H_2O$ soln; for chocolate drinks use 1.0 ml of soln contg 4.5 g $ZnSO_4.7H_2O$ and 0.1 g $CuSO_4.5H_2O$/100 ml; and for goat's milk use 1.0 ml of soln contg 7.5 g $ZnSO_4.7H_2O$ and 0.1 g $CuSO_4.5H_2O$/100 ml.) Mix thoroly (pH of mixt. should be 9.0–9.1.)

Step 7.—Filter (5 cm funnel, 9 cm Whatman No. 42 or No. 2 paper, or equiv.) and collect 5.0 ml filtrate in tube, preferably graduated at 5.0 and 10.0 ml.

Step 8.—Add 5.0 ml color development buffer, **15.054**(**a**)(2). (pH of mixt. should be 9.3–9.4.)

Step 9.—Add 4 drops BQC soln, **15.054**(**d**), mix, and let color develop 30 min. at room temp. (For merely detecting underpasteurization, add only 2 drops of the BQC soln.)

Step 10.—Det. intensity of blue color by one of following methods:

(*a*) *With photometer.*—Read color intensities of blank and test solns (using filter with max. transmittance ca 610 mμ), subtract reading of blank from that of test, and convert result to phenol equivs by reference to std curve, **15.054**(**g**)(2). [Ordinarily butyl alcohol extn is unnecessary when photometer is used; if butyl alcohol extn is made as in (*b*), centrifuge sample 5 min. to break emulsion and remove H_2O suspended in alcohol layer. (Babcock centrifuge can be adapted for this purpose by making special tube holders as follows: Slice section ¼″ thick from rubber stopper of suitable diam. to fit into bottom of centrifuge cup. Glue together 2 cork stoppers of appropriate diam., bore thru center a hole of proper size to hold tube snugly, and insert double cork section into cup.) After centrifuging, remove nearly all butyl alcohol by pipet with rubber bulb on top end. Filter into photometer cell and read with filter with max. light transmittance in region of 650 mμ.]

(**b**) *With visual standards.*—With samples yielding >5 units, compare colors in tubes with those of aq. phenol stds, **15.054**(**g**)(2). For quant. results in borderline instances (*e.g.*, tests yielding 0.5–5 units of color), ext. with butyl alcohol, **15.054**(**f**). Add 5.0 ml of the alcohol and invert

tube slowly several times; centrifuge as in (*a*) if necessary to increase clearness of alcohol layer, and compare blue color with colors of phenol stds, **15.054**(g)(2), similarly treated.

Step 11.—In tests observed to be strongly positive during color development (*e.g.*, 20 units or more), in which 4 drops of the BQC soln may be insufficient to combine with all the phenol, pipet appropriate proportion of contents into another tube, dil. to 10.0 ml with the color diln buffer, **15.054**(a)(3), and add 2 drops more of the BQC soln. With each test, dil. and treat blank in same manner. If test on dild sample is still very strongly positive, dil. again in same manner until final color is within range of visual stds or photometer std curve. Allow 30 min. for color development after last addn of the BQC soln before making final reading. To correct reading for diln, multiply by 2 for 5+5 diln, by 10 for 1+9 diln, and by 50 for 1+9 diln followed by 2+8 diln, etc.

Step 12.—When using 1.0 ml sample and adding 11.0 ml reagents (total liquid 12.0 ml, 5.0 ml filtrate used), multiply value of reading by 1.2 to convert to phenol equivs/0.5 ml sample. (If desired, results may be converted to phenol equivs /1 ml by multiplying by 2.4.) Phenol equivs >2/0.5 ml indicate underpasteurization in cow's milk, chocolate drinks, buttermilk, and cheese whey; phenol equivs >1/1.5 ml indicates underpasteurization in goat's milk.

Notes: To test concd milk products, reconstitute product with H_2O to original concn of milk solids and test in manner specified for original product.

See also Notes in 15.162.

15.057 Vitamin D in Milk—*See* 39.115 39.129

CREAM

15.058 Collection of Sample—Procedure

Proceed as in **15.001.** Analyze sample promptly, preferably within 3 days after collection.

15.059 Preparation of Sample—Procedure

Immediately before withdrawing test portions, mix sample by shaking, pouring, or stirring (or use hand homogenizer) until it pours readily and uniform emulsion forms. If sample is very thick, warm to 30–35° and mix. In case lumps of butter have sepd, heat sample to ca 38° by placing in warm H_2O bath. (Temp. appreciably >38° may cause fat to "oil off," especially in case of thin cream.) Thoroly mix portions for analysis and weigh immediately. (In commercial testing for fat by Babcock method, it may be advisable to warm all samples to ca 38° in H_2O bath previous to mixing.)

15.060 Preparation of Sample of Pressurized Cream—Official

Place containers in ice cream cabinet or equiv. overnight to freeze contents. Release as much gas as possible from frozen contents thru nozzle, holding container upright. Open container, using can opener on non-returnable type or wrench on heavier, returnable type. Empty contents into tared 1 L jar of high-speed blender, and weigh to 0.1 g. Let thaw (complete thawing is not necessary). Beat to smooth, creamy liquid, keeping blender covered. Beat intermittently to prevent overheating sample and blender. (Process may require 15 min. "Butter" stage is intermediate, and beating must be continued until this stage is passed.) When sufficiently mixed, weigh blender jar and contents again. Calc. % loss in wt and apply this correction to subsequent detns. Weigh samples for fat, solids, sucrose, or other analyses, beating few sec. between withdrawals of samples.

15.061 Lactic Acid—Official—*See* **15.008–15.013**

15.062 Water-Insoluble Fatty Acids (*29*)—Official

Weigh 50 g prepd sample **15.059,** into 250 ml centrifuge bottle, add 20 ml alcohol, shake, and add 50 ml ether. Proceed as in **15.125,** par. 2, except to use 10 ml of the Na_2WO_4 soln. (When Na_2WO_4-treated mixt. is shaken with ether, emulsions may form and not break completely on centrifuging. These emulsions may be broken by adding 10–20 ml alcohol, mixing gently, and again centrifuging.)

15.063 *Rapid Method—First Action—See* **15.133**

15.064 Volatile Acids—Official

Weigh 100 g sample into 250 ml vol. flask, add 100 ml H_2O and 2 ml H_2SO_4 (1+1), and mix, avoiding violent shaking. Add 15 ml *10% $Na_2WO_4.2H_2O$ soln,* dil. to mark, mix, and filter thru rapid paper. Transfer 150 ml filtrate to distn flask and proceed with distn, chromatographic sepn, and detn as in **18.019–18.021.** Calc. to mg acids/100 g fat.

15.065 Total Solids—Official

Proceed as in **15.014,** using 2–3 g sample.

15.066 Added Water (*18*)—Official

Proceed as in **15.039.**

15.067 Ash—Official—*See* **15.016**

15.068 Total Nitrogen—Official—*See* **15.017**

Lactose

15.069 *Gravimetric Method—Official—* *See* **15.028**

Fat

15.070 *Roese-Gottlieb Method—Official*

Using 5 g sample and dilg with H_2O to ca 10.5 ml, proceed as in **15.029**, beginning "Add 1.25 ml NH_4OH . . ."

Babcock Method—Official

15.071 APPARATUS

(**a**) *Test bottles.*—Std Babcock cream-test bottles are as follows:

(1) *50%, 9 g, short-neck, 6″ cream-test bottle.*—Total height 150–165 mm (5.9–6.5″). Bottom of bottle must be flat, and axis of neck must be vertical when bottle stands on level surface. Charge of cream for bottle is 9 g.

Bulb.—Capacity of bulb to junction with neck is not <45 ml. Shape of bulb may be either cylindrical or conical. If cylindrical, o. d. must be 34–36 mm; if conical, o. d. of base must be 31–33 mm, and max. diam., 35–37 mm.

Neck.—Cylindrical and of uniform diam. from at least 5 mm below lowest graduation mark to at least 5 mm above highest. Top of neck is flared to diam. of not <15 mm. Graduated portion of neck has length of not <63.5 mm. Total % graduation is 50. Graduations shall represent 5, 1, and ½%, resp., from 0.0 to 50%. 5% graduations must extend at least half-way around neck to right; ½% graduations must be not <3 mm long; and 1% graduations must be intermediate in length between 5% and ½% graduations and project 2 mm to left of ½% graduations. Each 5% graduation must be numbered (thus: 0, 5, 10, . . . 45, 50), number being placed to left of scale. Capacity of neck for each whole % on scale must be 0.1 ml. Max. error in total graduation or any part thereof must not exceed vol. of smallest unit of graduation.

(2) *50%, 9 g, long-neck, 9″ cream-test bottle.*—Same specifications apply to this bottle as to 50%, 9 g, 6″ cream-test bottle, except that total height of this bottle is 210–229 mm (8.25–9.0″) and graduated portion of neck has length of not <120 mm.

(3) *50%, 18 g, long-neck, 9″ cream-test bottle.*—Same specifications apply to this bottle as to 50%, 9 g, 9″ cream-test bottle, except that charge of cream for this bottle is 18 g.

Each bottle must bear on top of neck above graduations, in plain legible characters, mark denoting wt charge to be used, *viz.*, "9 g" or "18 g," as case may be.

Each bottle must be constructed so as to withstand stress to which it will be subjected in centrifuge.

(4) *Testing.*—Proceed as in **15.030**(**a**)(3).

(**b**) *Water bath for cream samples.*—Provided with thermometer and device to maintain temp. of 38°.

(**c**) *Cream weighing scales.*—With sensibility reciprocal of 30 mg, *i.e.*, addn of 30 mg to either pan of scale, when loaded to capacity, causes deflection of at least 1 subdivision of graduation. Set scales level upon support and protect from drafts.

(**d**) *Weights.*—9 g and 18 g, resp., and plainly marked "9 g" or "18 g," as case may be. Must be made of material capable of resisting corrosion or other injury, and preferably of low squat shape, with rounded edges. Verify them at frequent intervals by comparison with stdzd wts.

(**e**) *Acid measure.—See* **15.030**(**c**).

(**f**) *Centrifuge or "tester."—See* **15.030**(**d**).

(**g**) *Dividers or calipers.—See* **15.030**(**e**).

(**h**) *Water bath for test bottles.—See* **15.030**(**f**).

15.072 DETERMINATION

Weigh 9 g prepd sample, **15.059,** directly into 9 g cream-test bottle, or 18 g into 18 g bottle, and proceed by one of following methods.

(**a**) *Method 1.*—After weighing cream into test bottle, add 8–12 ml H_2SO_4 (sp. gr. 1.82–1.83 at 20°) to 9 g bottle; or 14–17 ml to 18 g bottle; or add acid until mixt. of cream and acid, after shaking, has chocolate-brown color. Shake until all lumps completely disappear and add 5–10 ml soft H_2O at 60° or above. Transfer bottle to centrifuge, counterbalance it, and after proper speed is reached, whirl 5 min. Add soft hot H_2O until liquid column approaches top graduation of scale; then whirl 1 min. longer at 55–60°. Adjust temp. as in **15.031,** and with aid of dividers or calipers measure fat column, in terms of % by wt, from lower surface to bottom of upper meniscus.

(**b**) *Method 2.—For 9 g bottle only.*—After weighing cream into test bottle, add 9 ml soft H_2O and mix thoroly; add ca 17.5 ml of the H_2SO_4 and shake until all lumps completely disappear. Transfer bottle to centrifuge, counterbalance it, and after proper speed is reached, whirl 5 min. Fill bottle to neck with hot H_2O and whirl 2 min. Add hot H_2O until liquid column approaches top graduation of scale, and whirl 1 min. longer at 55–60°. Adjust temp. and measure fat column as in (**a**).

Whichever method is followed, fat column, at time of reading, should be translucent, golden yellow to amber, and free from visible suspended particles. Reject all tests in which fat column is

milky or shows presence of curd or of charred matter, or in which reading is indistinct or uncertain; repeat test, adjusting quantity of H_2SO_4 added.

If glymol or pure white mineral oil (sp. gr. not >0.85 at 20°) is used, introduce only few drops into bottle just before reading is made, letting it flow down inside of neck. For purpose of measurement, surface sepg glymol and fat is regarded as representing upper limit of column. Oil-sol. artificial color may be added to the white mineral oil.

15.073 Gelatin—Official—*See* 15.040
Observe note

15.074 Preservatives—Official—*See* 15.041 and Chap. 27

15.075 Coloring Matters—Official—*See* 15.045 and Chap. 35

Residual Phosphatase

15.076 *Method I.—Official—See* **15.047–15.053**

Method II. (17)—Official

15.077 REAGENTS AND SAMPLING—*See* **15.054 and 15.055**

15.078 DETERMINATION

Proceed as in **15.056**, except for following:

In *Step 1*, use 1.0 g instead of 1 ml sample if desired.

In *Step 3*, treat fresh cream in same manner as fresh milk; for old or slightly sour cream use 8 ml of the Ba borate-hydroxide buffer, **15.054**(a)(1), and 2 ml H_2O.

In *Step 6*, treat fresh cream in same manner as fresh milk; for old or slightly sour cream substitute 1.0 ml 4.5 g/100 ml soln $ZnSO_4.7H_2O$ for the Zn-Cu precipitant.

Phenol equivs $>2/0.5$ ml or 0.5 g indicate underpasteurization.

EVAPORATED MILK (UNSWEETENED)

15.079 Preparation of Sample—Procedure

(**a**) Temper unopened can in H_2O bath at ca 60°. Remove and shake can vigorously every 15 min. After 2 hr, remove can and let cool to room temp. Remove entire lid and thoroly mix by stirring contents in can with spoon or spatula. (If fat seps, sample is not properly prepd.)

(**b**) Dil. 40 g prepd mixt. (a) with 60 g H_2O and mix thoroly.

15.080 Lactic Acid—Official—*See* 15.008–15.013

15.081 Total Solids (*4*)—Official

Proceed as in **15.014**, using 4–5 g dild sample, **15.079**(b). Correct result for diln.

15.082 Ash (*4*)—Official

Ignite residue from total solids detn, **15.081**, at temp. not $>550°$ until ash is C-free. Correct result for diln.

15.083 Fat—Official

Weigh 4–5 g undild sample, **15.079**(a), into Mojonnier fat-extn flask or Röhrig tube; dil. with H_2O to ca 10.5 ml; and proceed as in **15.029**, beginning "Add 1.25 ml NH_4OH . . . " Some evapd milks may require centrifuging for as long as 20 min. at 600 rpm for complete sepn of emulsion.

15.084 Total Nitrogen—Official

Weigh 5 g undild sample, **15.079**(a), transfer to Kjeldahl flask, and proceed as in **2.036**. % $N \times 6.38 = \%$ "protein."

15.085 Casein—Official

Weigh 10 g dild sample, **15.079**(b), into beaker, and proceed as in **15.018** or **15.020**. Correct result for diln.

15.086 Albumin—Official

Proceed as in **15.021**, using filtrate from casein detn, **15.085**. Correct result for diln.

15.087 Lactose—Official

Proceed as in **15.027** or **15.028**, using dild sample, **15.079**(b), and correct result for diln.

15.088 Gelatin—Official—*See* 15.040

15.089 Preservatives—Official—*See* 15.041 and Chap. 27

15.090 Coloring Matters—Official—*See* 15.045 and Chap 35

SWEETENED CONDENSED MILK

15.091 Preparation of Sample—Procedure

(**a**) Temper unopened can in H_2O bath at 30–35° until warm. Open, scrape out all milk adhering to interior of can, transfer to dish large enough to permit stirring thoroly, and mix until whole mass is homogeneous.

(**b**) Weigh 100 g thoroly mixed sample into 500 ml vol. flask, dil. to mark with H_2O, and mix thoroly. If sample will not emulsify uniformly, weigh out sep. portion of (a) for each detn.

15.092 Lactic Acid—Official—*See* 15.008–15.013

15.093 Total Solids—Official

Transfer 10 ml prepd soln, **15.091**(b), to weighed flat-bottom dish, not <5 cm diam., contg 15–20 g dry sand or asbestos fiber. Heat on steam bath 30 min. and then in vac. oven at 100°

to constant wt. Cool in desiccator and weigh quickly to avoid absorption of H_2O. Correct result for diln.

15.094 Ash—Official

Evap. 10 ml prepd soln, **15.091(b)**, to dryness on H_2O bath and ignite residue as in **29.012** or **29.013.** Correct result for diln.

15.095 Fat—Official

Weigh accurately 4–5 g prepd sample, **15.091(a)**, into Mojonnier fat-extn flask or Röhrig tube; dil. with H_2O to ca 10.5 ml, and proceed as in **15.029**, beginning "Add 1.25 ml NH_4OH . . ."

15.096 Protein—Official

Det. N as in **2.036**, using 10 ml prepd soln, **15.091(b)**, and correct result for diln. % N×6.38 = % total "protein."

15.097 Lactose—Official

Dil. 100 ml prepd soln, **15.091(b)**, in 250 ml vol. flask to ca 200 ml; add 6 ml $CuSO_4$ soln, **29.035(a)**, and alkali soln of concn and in proportion as in **15.028.** Dil. to mark and mix thoroly. Filter thru dry filter and det. lactose as in **29.039.** Correct result for diln.

Sucrose (*19*)–Official

15.098 REAGENT

Mercuric nitrate soln.—To 220 g yellow HgO, add 300–400 ml H_2O and enough (but with min. excess) HNO_3 to form clear soln (ca 140 ml), being careful to use least possible excess of acid. Dil. to 800–900 ml and add 10% NaOH soln slowly and with constant shaking until slight permanent ppt forms. Dil. to 1 L and filter. As soln tends to become acid with age owing to deposition of basic Hg salts, add dil. alkali occasionally until slight permanent ppt forms and refilter.

15.099 DETERMINATION

Place 50 ml prepd soln, **15.091(b)**, in 100 ml vol. flask; add 25 ml H_2O, mix, add 5 ml of the $Hg(NO_3)_2$ soln, and shake thoroly. Without delay and while shaking constantly, neutralize to litmus paper with $0.5N$ NaOH, but avoid alk. reaction (12–13 ml). Dil. to 100 ml with H_2O, mix thoroly, and filter thru dry paper. Polarize filtrate in 200 mm tube; then invert at room temp. as in **29.026(c)** and polarize inverted soln. Correct both readings for vol. occupied by protein, **15.096**, and fat, **15.095**; 1 g protein occupies 0.8 ml and 1 g fat, 1.075 ml. Calc. % sucrose by following formula, using corrected direct and invert readings obtained above:

$$S = \frac{100(a - b)}{142.35 - \frac{t}{2}} \times \frac{26}{W},$$

where S = % sucrose in sample; a = corrected direct polarization; b = corrected invert polarization; t = temp. of soln polarized; and W = wt sample taken (10 g).

DRIED MILK AND MALTED MILK

15.100 Sampling Dried Milk (*20*)—Procedure

Avoid sampling on rainy day or when humidity is high so as to reduce moisture absorption from air to min.

On surface of milk at top of barrel locate point on each end of a diam. and on radius perpendicular to this diam., 1–2″ in from edge of barrel. Midway on each side of triangle between these points locate one point. At 6 points so located, using tubular trier long enough to extend full length of barrel, draw core parallel to vertical axis of barrel. Transfer cores to clean, dry, air-tight container and seal immediately.

Before opening sample for analysis, make homogeneous either by shaking or by alternately rolling and inverting container. Avoid excessive temp. and humidity when opening sample container.

15.101 Preparation of Sample—Procedure

Sift sample thru No. 20 sieve onto large sheet of paper, rubbing material thru sieve and tapping vigorously if necessary. Grind residue in mortar, pass thru sieve, and mix into sifted material. Discard particles of wood and other material that cannot be ground. Sift sample 2 more times, mixing thoroly each time. To avoid absorption of moisture, operate as rapidly as possible, and preserve sample in air-tight container.

15.102 Moisture (*21*)—Official

Weigh 1–1.5 g sample into round, flat-bottom metal dish (not <5 cm diam. and provided with tight-fitting slip-in cover). Loosen cover and place dish on metal shelf (dish resting directly on shelf) in vac. oven kept at temp. of boiling H_2O. Dry to constant wt (ca 5 hr) under pressure not >100 mm (4″) of Hg. During drying admit slow current of air into oven (ca 2 bubbles/sec.), dried by passing thru H_2SO_4. Stop vac. pump and carefully admit dried air into oven. Press cover tightly into dish, remove from oven, cool, and weigh. Calc. % loss in wt as moisture.

15.103 Protein—Official

Weigh 1 g sample into Kjeldahl digestion flask and det. N as in **2.036.** % N×6.38 = % "protein."

15.104 Casein in Malted Milk and Chocolate Malted Milk (*22*)—Official

Place 10 g sample in 250 ml (or larger) centrifuge bottle and ext. with two 100 ml portions petr. ether by shaking until uniform, centrifuging, and decanting supernatant. To dry residue add exactly 200 ml *3% $Na_2C_2O_4$ soln.* Shake occasionally over 4 hr period. Centrifuge 15 min. at high speed (1800 rpm if Size 1 type SB centrifuge is used). Pipet 50 ml supernatant (100 ml for chocolate malted milk product) into 250 ml beaker. Add 50 ml paper pulp suspension (1 filter paper) and 2 ml HOAc dropwise with constant stirring. Set beaker in 45–50° H_2O 15 min. Cool to room temp. and filter with moderate suction thru 7 cm büchner, previously fitted with No. 589 S&S white ribbon paper or equiv. and overlaid with layer of paper pulp. Wash ppt 2 or 3 times with cold H_2O. (Filtrate should be clear, or nearly so. If first portions of filtrate are not clear, repeat filtration and finish washing ppt.) Det. N in washed ppt and filter paper as in **2.036,** and multiply by 6.38 to obtain equiv. casein. Correct result for blank on reagents and paper pulp.

15.105 Ash—Official

Ignite 1 g sample at 550° until C-free. If suitable dish was used for moisture detn, **15.102,** ash may be detd on same portion. Cool in desiccator and weigh.

15.106 Alkalinity of Ash in Dry Skim Milk (*23*)—Official

Ash 2 g dry skim milk 1 hr at 550°. Add few ml H_2O to ash, break up with flat-end stirring rod, evap. to dryness over steam bath, again ash 1 hr, and weigh. Again add few ml H_2O to ash, break up, and transfer to beaker with 50–75 ml H_2O. Add 50 ml 0.1N HCl, heat to boiling, and boil gently 5 min. Cool, add 30 ml *40% $CaCl_2$ soln* (neutralized with 0.1N HCl and filtered) and ca 10 drops phthln, and titr. excess acid with 0.1N NaOH. Acid used (ml)$\times$50 = alky of ash.

15.107 Fat in Malted Milk (*24*)—Official

Weigh quickly ca 1 g well-mixed sample into small, lipped beaker. Add 1 ml H_2O and rub to smooth paste. Add 10 ml more of H_2O, warm on steam bath, and transfer to Mojonnier fat-extn flask or Röhrig tube with 10 ml alcohol. Mix thoroly, cool, and proceed as in **15.029,** beginning "Add 25 ml ether . . . " rinsing beaker with this ether.

Fat in Dried Milk (*25*)—Official

15.108 PREPARATION OF SOLUTION

Proceed as in one of following methods:

(**a**) Weigh quickly ca 1 g well-mixed sample into small beaker. Add 1 ml H_2O and rub to smooth paste. Add 9 ml more of H_2O and 1 ml NH_4OH, and warm on steam bath. Transfer to Mojonnier fat-extn flask or Röhrig tube. Cool, and proceed as in **15.109,** rinsing beaker successively with the alcohol and ethers used in first extn.

(**b**) Weigh quickly ca 1 g well-mixed sample and transfer to Mojonnier fat-extn flask or Röhrig tube. Add 10 ml H_2O and shake until homogeneous, warming if necessary. Add 1 ml NH_4OH and heat in H_2O bath 15 min. at 60–70°, shaking occasionally. Cool, and proceed as in **15.109.**

15.109 DETERMINATION

Add 10 ml alcohol to sample and mix. Ext. with ether and petr. ether as in **15.029.** For second extn add 4 ml alcohol, and again ext. as in **15.029.** With whole milk and cream powders make third extn, using 15 ml of each solvent after adding, if necessary, enough H_2O to bring aq. layer in tube to original vol.

15.110 Citric Acid in Dried Milk (*2*)—Official

Weigh 6 g well-mixed sample, mix well with 44 ml H_2O, and proceed as in **15.005,** beginning "add ca 100 mg *tartaric acid* . . . "

15.111 Lactic Acid (*3*)—Official—*See* 15.008–15.013

BUTTER

(Methods are also applicable to renovated or process butter and margarine)

15.112 Sampling (*26*)—Procedure

(**a**) *Tub or cube butter.*—Insert regular trough butter trier practically its full length from point near top edge (or corner in case of cube) thru center to point at bottom diagonally opposite point of entry. Make one complete turn and withdraw full core. Hold point of trier over mouth of sample container and immediately transfer core of butter in ca 3″ sections, working it from trier by aid of spatula fitted to groove. Leave plug ca 1″ long to place in hole from which core was removed. Add 2 other trierfuls taken similarly at points equidistant from first (2 other corners in case of cube) to the jar to constitute subdivision from tub or cube sampled. Do not include moisture adhering to outside of trier. Clean and dry trier before each drawing. Use unwarmed trier for butter stored above f.p. For harder butter use trier warmed to temp. that may be just borne

by hand. Soften butter frozen so hard as to resist trier by storage in tempering room 24 hr.

Sample lots as follows:

(1) *Tubs (or cubes) marked with churn numbers.*—Sample 1 tub of each churn of 1–9 tubs, 2 of each churn of 10–14 tubs, and 3 of each churn of >14 tubs. In no case sample <2 tubs in lot.

(2) *Tubs (or cubes) not marked with churn numbers.*—Sample number of tubs equiv. to square root of number in lot, with min. of 3 and max. of 25. If square root is not whole number, sample 1 extra tub.

(**b**) *Print butter.*—Withdraw 1 print from each of number of cases equiv. to square root of number of cases in lot, with min. of 5 and max. of 25. When square root is not whole number, sample 1 extra case. Select cases to include each churn or batch mark when so marked. With <5 cases sample all, taking 5 prints as min. Remove wrapper and transfer each print to sep. sample container. (With prints of 1 lb or over, print may be quartered and 2 opposite quarters selected as sample.) With 8 oz and 4 oz prints, take whole print as sample.

These directions provide min. sampling, to be increased if object of examination demands.

(**c**) *Sample containers.*—Use glass jar of such type as to prevent loss of moisture by evapn or entrance of H_2O into jar. (Do not use jar tops contg liner of any material.)

Preparation of Sample *(27)*

15.113 *Shaking Method—Official*

Soften entire sample in sample container, **15.112(c)**, by warming in H_2O bath kept at as low temp. as practicable, not >39°. Avoid overheating, which causes visible sepn of curd. Shake at frequent intervals during softening process to reincorporate any sepd fat, and observe fluidity of sample. Optimum consistency is attained when emulsion is still intact but fluid enough to reveal sample level almost immediately. Remove from bath and shake vigorously at frequent intervals or place sample container in mechanical shaking machine that simulates hand shaking, with arm 9″ long, set to oscillate at 425 ± 25 times/min. thru arc of 1.75″. Continue shaking until sample cools to thick, creamy consistency and sample level can no longer readily be seen. Weigh portion for analysis promptly.

15.114 **Moisture—Official**

Weigh 1.5–2.5 g prepd sample, **15.113**, into flat-bottom dish not <5 cm diam. and dry to constant wt in oven kept at temp. of boiling H_2O. Clean, dry sand or asbestos may be used if fat is not to be detd in residue by **15.115**.

Fat *(27)*

15.115 *Indirect Method—Official*

Take up dry butter obtained in moisture detn in which no absorbent was used, **15.114**, by macerating with 15 ml absolute ether or petr. ether; transfer to weighed gooch with aid of wash bottle filled with the solvent; and wash free from fat with 100 ml solvent. (Pass last 25 ml solvent thru crucible without suction.) Dry crucible and contents at temp. of boiling H_2O to constant wt. Repeat washing with 25 ml solvent and dry to constant wt. Repeat operation until there is no loss in wt due to washing. % fat = 100 − (% moisture + % residue).

15.116 *Direct Method—Official*

From dry butter obtained in detn of moisture either with or without use of absorbent, ext. fat with anhyd., alcohol-free ether or petr. ether (b.p. <65°), receiving soln in weighed flask. Evap. solvent and dry ext. to constant wt at temp. of boiling H_2O.

15.117 **Casein, Ash, and Salt—Official**

Cover crucible contg residue from fat detn by indirect method, **15.115**; heat, gently at first, and gradually raise temp. to not >500°. Remove cover and continue heating until residue is white. Loss in wt represents casein; residue in crucible represents mineral matter. Dissolve residue in H_2O slightly acidified with HNO_3 and det. Cl, either gravimetrically as in **6.066**, or volumetrically as in **6.068**, and calc. % NaCl.

15.118 **Salt—Official**

Weigh 5–10 g sample in counterpoised beaker. Add ca 20 ml hot H_2O, and after butter melts, transfer to separator. Insert stopper and shake few min. Let stand until all fat collects on top of the H_2O; then drain H_2O into flask, being careful to let none of fat globules pass. Again add hot H_2O, rinsing beaker, and repeat extn 10–15 times, using 10–20 ml H_2O each time. Washings will contain all but mere trace of NaCl originally present in butter. Det. quantity in whole or in aliquot of liquid by titrn with std $AgNO_3$ soln, **31.009(b)**, using K_2CrO_4 indicator, **31.009(a)**.

15.119 **Examination of Fat—Official**

Melt butter and keep 2–3 hr in dry place at ca 60°, or until H_2O and curd sep. completely. Filter clear supernatant fat thru dry paper in hot H_2O funnel or in oven at ca 60°. If filtered liquid fat is not perfectly clear, refilter. Det. physical and chemical constants as in Chap. 26, particularly mole per cent butyric acid, **26.028 26.033**, cholesterol and phytosterol in mixtures of animal and vegetable fats, **26.045 26.047**, and soluble

and insoluble volatile acids (Reichert-Meissl and Polenske values), 26.026–26.027.

Critical Temperature of Dissolution (*28*)—Official

15.120 REAGENT

Alcohol iso-amyl alcohol reagent.—Mix 2 vols 95% (by vol.) alcohol (checked by sp. gr.) with 1 vol. redistd isoamyl alcohol (b.p. 128–132°) both measured with pipet or vol. flask. Keep well-stoppered.

15.121 APPARATUS

(a) *Test tubes.*—Pyrex, 18×150 mm, marked at 2 and 4 ml, measured by adding H_2O from buret.

(b) *Micro burner.*

(c) *Pipet.*—Glass tube, ca 2–3 ml capacity, drawn to fast-flowing tip.

(d) *Thermometer.*—Range 0–100°, graduated in degrees.

15.122 DETERMINATION

Prep. oil from butter or margarine as in **15.119.** Oil must be clear. Fill test tube to 2 ml mark with oil, using pipet. Immediately add alcohol reagent to 4 ml mark (or add 2 ml with pipet). Using thermometer as stirring rod, mix the two layers and heat in flame of micro burner. Keep stirring and heating until mixt. becomes clear and homogeneous. *Do not boil.* Remove from heat and keep stirring until definite turbidity appears in *mixture proper.* Record temp. at first discernible turbidity. (Opalescence will immediately follow thruout entire mixt. with further drop in temp.)

15.123 Coloring Matters—Official

Pour ca 2 g filtered fat, dissolved in ether, into each of 2 test tubes. To one tube add 1–2 ml HCl (1+1) and to other ca same vol. 10% NaOH soln. Shake tubes well and let stand. In presence of some azo dyes, acid soln shows pink to wine-red color, while alk. soln in other tube shows no color. If, on other hand, annatto or other vegetable color is present, alk. soln is colored yellow, while no color is apparent in acid soln. (Red color changing to yellow, especially on warming, in alk. soln, may be due to presence of gallate antioxidants.)

General test.—Proceed as in Chap. **35** for detection of oil-sol. coal-tar dyes and annatto.

15.124 Lactic Acid—Official—*See* 15.008–15.013

Water-Insoluble Fatty Acids (WIA)

15.125 *Gravimetric Method (29)—Official*

Weigh 50 g prepd sample, **15.113,** into 250 ml centrifuge bottle, and add 10 ml H_2O; if necessary, remelt in warm H_2O (not steam) bath and add 50 ml ether. Shake until fat dissolves.

Add 1*N* NaOH in ca 0.2 ml increments to mixt. in centrifuge bottle until neutralized to decided pink, using 10 drops phthln, and shaking between addns of alkali. Then add 0.5 ml in excess and shake again at least 2 min. During this and all subsequent shakings release pressure carefully several times to avoid blowing out stopper and losing some of contents. (It is difficult to shake >1 bottle at a time because of greasy stoppers and pressure that develops.)

Remove stopper and add 50 ml petr. ether, shake few times, and centrifuge 5 min. at ca 1200 rpm (longer if sepn is not sharp). Set bottle on horizontal surface and siphon off ether-fat layer. (If ether layer, after centrifuging, is reddish, add 10 ml H_2O, shake, and again centrifuge as before. If reddish color still persists in ether layer, add 25 ml ether, shake, and again centrifuge.) Wash aq. layer remaining in centrifuge bottle by adding 25 ml ether; mix thoroly by shaking several sec., add 25 ml petr. ether, and again mix by shaking. Centrifuge, siphon off ether layer as before, and repeat washing as above. If after any washing, basic red color of phthln fades, add addnl phthln and alkali to give decided red (not pink).

Add 1 ml H_2SO_4 (1+1) to residue in centrifuge bottle and shake vigorously few sec. Then add 5 ml *10% $Na_2WO_4.2H_2O$ soln* and again shake vigorously few sec. (Mixt. should be distinctly acid to *congo red paper;* if it is not, add more of the H_2SO_4.) Now add 75 ml ether, shake violently at least 2 min., and centrifuge. Siphon off ether layer into 500 ml separator. Wash siphon inside and out with 75 ml ether so that washings drain into centrifuge bottle. Shake bottle violently at least 2 min., centrifuge, and siphon off ether layer into the separator. (Disregard slight opalescence of ether layer.)

Add 100 ml alcohol (1+1) to combined exts in separator and neutralize in same manner as before with 1*N* NaOH to decided pink. Add 0.5 ml excess and shake violently 2 min. more. Immediately add 25 ml H_2O, mix by single inversion of separator, and let stand until aq. layer is clear. (Sepn usually occurs in few min.; slow sepn may sometimes be hastened by playing fine stream of H_2O on ether surface. If vol. of emulsion at interface is only ca 10 ml it may be included in subsequent extn.) Drain aq. layer into 600 ml beaker. Add 50 ml of the 1+1 alcohol and ca 10 drops phthln to contents of separator and neutralize with the alkali, shaking vigorously ca 2 min. Add 50 ml H_2O, mix by single inversion of separator, and let stand until aq. layer is clear. Drain aq. layer into the beaker. Then add 10 ml H_2O to separator, mix by single inversion, let sep. until aq. layer is clear, and drain into beaker.

Place beaker contg combined exts and washings on steam bath (or carefully heat on hot plate) to expel any ether. Evap. to ca 25 ml (small fan is useful if foaming is serious). (Decided red color should persist thru all these operations and up to point where soaps are acidified.) Transfer to 250 ml beaker with ca 25 ml H_2O. (As alternative procedure material may be evapd to dryness on steam bath and residue dissolved in ca 50 ml H_2O.)

Dissolve 5 g anhyd. Na_2SO_4 in the warm soln (vol. must be not <50 ml when Na_2SO_4 and H_2SO_4 are added), heating if necessary. Cool to 20° or lower, stirring frequently to keep soaps from forming hard crust on surface. Make acid by adding H_2SO_4 (1+1) dropwise, using congo red paper as indicator. Stir vigorously to effect thoro liberation of fatty acids, mashing all pink soap curds. Add ca 500 mg filter-aid and mix. Filter with suction on suitable filter. Rinse beaker with three ca 15 ml portions H_2O at 20° or lower and transfer rinsings to crucible. Maintain suction *several min. after visible dripping ceases* to dry ppt. (Heavy ppts can be sucked drier if cracks are plastered up with some of ppt. Filtrate should be clear.)

Substitute tared beaker or flask (weighed with similar vessel as counterpoise), contg few glass beads or grains of sand, for receiving flask of filtering app. Ext. acids with four ca 15 ml portions ether, breaking up ppt with stirring rod between extns and thoroly mixing with the ether. (Filter pad must not be disturbed.) Evap. ether ext., which should be no more than faintly opalescent, on steam bath, and dry acids in 100° oven 1 hr. Cool and weigh. Report results as mg H_2O-insol. acids (WIA)/100 g butterfat.

Dissolve weighed acids in 10 ml neutral benzene and titr. with 0.1N Na ethylate (prepd similarly to 0.05N Na ethylate, **16.032(b)**), using 10 drops phthln as indicator, until end point holds at least 1 min. (Neutral alcohol instead of benzene and 0.1N NaOH instead of Na ethylate may be used.) Compute mean molecular wt of fatty acids by dividing mg acids found by ml 0.1N alkali used for titrn and multiplying by 10. (Mean molecular wt should not be >290. When quantity of acids is <150 mg/100 g butterfat, mean molecular wt is without significance.)

NOTES: To siphon off ether use tube similar to delivery tube of ordinary wash bottle but with intake end bent up into U shape in opposite direction to outlet end, with opening ¼–½" higher than bottom of U, cut off horizontally. (Avoid excessive constriction when bending.) Set delivery tube loosely enough in stopper that it can be raised or lowered. In operating, adjust opening of U bend to ca ⅛" above surface of aq. layer and blow ether layer off by gently blowing thru mouthpiece tube inserted in adjacent hole in stopper.

Following setup is convenient for filtration of fatty acids: Bell jar and gooch with removable bottom charged with thin layer of asbestos overlaid with small quantity of filter-aid (Dicalcite Co.'s "Speedex" added as suspension in H_2O). Use long fiber, amphibole variety, acid and alkali-washed asbestos for gooch, washed twice by decantation. (Coarse fritted glass crucibles overlaid with small quantity of filter-aid are also satisfactory.)

Water-Insoluble Fatty Acids (WIA) and Butyric Acid

Chromatographic Method—First Action

15.126 APPARATUS

Chromatographic column.—Approx. 2×40–50 cm. Tube described in **26.028** is also satisfactory.

15.127 REAGENTS

(a) *n-Hexane-butanol soln.*—*n*-Hexane contg 1% *n*-butanol. *See* **26.029(d)**.

(b) *Silicic acid.*—Mallinckrodt's powder especially prepd for chromatography. Dry 2 hr at 130° and keep in tightly stoppered bottles.

(c) *Ammoniacal glycol soln.*—Dissolve 500 mg bromocresol green in 500 ml ethylene glycol by warming on steam bath. Cool, add NH_4OH dropwise until soln is dark blue (1–3 drops), and then add 1 drop excess. This soln should give olive-green color when mixed with the silicic acid.

(d) *Thymol blue soln.*—Dissolve 300 mg thymol blue in 10 ml of the 0.05N alc. KOH and add 90 ml isopropyl alcohol. Soln should be blue; if not, add enough 0.05N alc. KOH to make soln dark blue.

(e) *Alcoholic KOH soln.*—0.05N. Dissolve 4 g KOH pellets in 100 ml isopropyl alcohol by warming and swirling on steam bath. Decant supernatant from small amount of aq. KOH soln clinging to flask. Dil. to 1 L with 400 ml isopropyl alcohol and 500 ml alcohol. Stdze with NBS benzoic acid, using 2 drops thymol blue as indicator.

(f) *Cresol red indicator.*—Dissolve 50 mg *o*-cresolsulfonphthalein in 20 ml alcohol. Add 1.3 ml 0.1N NaOH and dil. to 50 ml with H_2O.

15.128 PREPARATION OF SAMPLE

Weigh 100 g prepd sample, **15.113**, into 250 ml beaker. Melt on steam bath and transfer to 500 ml separator. Rinse beaker with 20 ml H_2O and then with 100 ml ether, and add rinsings to separator. Shake until fat dissolves.

Add 1N NaOH in ca 0.2 ml increments to mixt. in separator until neutralized to decided pink, using 10 drops phthln as indicator and shaking between addns of alkali. Then add 0.5 ml excess and shake again at least 2 min. Add 100 ml petr. ether, shake few times, and let layers sep. (5–10 min.). Drain aq. layer and any emulsion into 250 ml centrifuge bottle. Shake fat layer with 10 ml H_2O and add aq. layer to centrifuge bottle after

sepn. Wash soln in centrifuge bottle by adding 50 ml ether, mixing thoroly by shaking; add 50 ml petr. ether and again mix by shaking. Centrifuge 5 min. at ca 1200 rpm and siphon off ether-fat layer. If sepns have not been sharp and if much fat is still present, repeat washing with 50 ml ether and 50 ml petr. ether. Continue as in **15.125**, beginning, 4th par., "Add 1 ml H_2SO_4 (1+1) . . . ", except use 10 ml 10% $Na_2WO_4.2H_2O$ instead of 5 ml and two 100 ml portions ether instead of 75 ml. When material has evapd to 25 ml on hot plate or steam bath, transfer to 100 ml beaker and evap. to dryness on steam bath or, with extreme care, on hot plate.

15.129 PREPARATION OF CHROMATOGRAPHIC COLUMN

To 20.0 g silicic acid in mortar add 14 ml ammoniacal glycol soln. Mix thoroly with pestle until homogeneous. Add few ml of the hexane-butanol soln and mix to form smooth paste; then add enough solvent to form slurry. Add slurry with spoon thru funnel to chromatographic column contg small, loosely-packed cotton plug in constricted end, and ca 30 ml hexane-butanol soln. Force excess solvent thru column, using pressure of 5–10 lb/sq. in., **18.015(c)**. Remove pressure when all of solvent has passed into gel but before column "cracks." Keep small amount of solvent on top of column until ready for use. Do not use cracked column.

15.130 SEPARATION OF FATTY ACIDS

To dry residue of Na salts in 100 ml beaker, **15.128**, add 0.50 ml H_2O and mix well. Add 0.50 ml H_2SO_4 (1+1) and mix well, being careful to break up and neutralize all lumps. (Sharp needle or stirring rod is sometimes needed to scrape material from bottom of beaker.) Add 2.0 g silicic acid and stir to uniform powder.

Add enough solvent to top of column to make 5 ml. Dry sides of column by stream of air from glass tube and rubber bulb. Transfer powder to liquid in column. Mix powder and solvent on top of column with long stirring rod, being careful not to disturb surface of original gel. Force excess solvent into column with pressure. Rinse stirring rod, funnel, beaker, and sides of column with two 10 ml portions solvent, forcing excess solvent into column after each addn.

Fill column with solvent and force solvent thru column dropwise (2–3 ml/min.) with as much pressure as necessary. If column cracks or solvent flows thru column too rapidly, prep. new column, reducing amount of ammoniacal glycol soln added to silicic acid (use ca 1 ml less). If solvent flows too slowly even with pressure, use more ammoniacal glycol soln.

Collect first 125 ml eluate and titr. with std 0.05N alc. KOH, using 10 drops thymol blue soln as indicator. 1 ml 0.05N KOH = 13.5 mg WIA. Discard next 40–60 ml or until yellow band of butyric acid starts to elute. Collect next 75 ml eluate and titr. with 0.01N NaOH, using 25 ml *CO_2-free, neutralized H_2O* and 2–3 drops cresol red indicator. 1 ml 0.01N NaOH = 0.88 mg butyric acid. Calc. WIA and butyric acid to mg/100g fat.

Rapid Method (30)—First Action

(Does not recover salts of WIA produced by neutralization)

15.131 REAGENT

Sodium ethylate.—See **16.032(b)**. (MeOH or 95% alcohol may be substituted for absolute alcohol, and K may be substituted for Na.)

15.132 PREPARATION OF SAMPLE

(**a**) *Cream.*—Weigh 20 g into 125 ml g-s. erlenmeyer, ₮ No. 22. Add 25 ml ice-cold H_2O, cool to 10°, and shake until butterfat seps in granular form. Discard if the granular fat conglomerates into one lump.

(**b**) *Butter.*—Weigh 10 g into 125 ml g-s. erlenmeyer, ₮ No. 22, warm to melt butter, and cool until butter is of thick, creamy consistency. Add 50 ml ice-cold H_2O, shake, cool to 10°, and shake ca 5 sec.

15.133 DETERMINATION

Insert filter sieve, Fig. 29 (available from Langsenkamp-Wheeler Brass Works, Indianapolis, Ind.) into ₮ erlenmeyer and pour off serum layer. Add 50 ml ice-cold H_2O, insert glass stopper, and shake ca 5 sec. Pour off liquid thru inserted filter sieve. Wash 3 addnl times. Dissolve washed butter in 25 ml ether, pour into small separator, wash erlenmeyer with few ml ether, and add to separator. Let settle few min. and drain off aq. curd layer. Drain ether-fat soln into 125 ml erlenmeyer, wash separator with few ml ether, add to erlenmeyer, and titr. with the 0.05N Na ethylate, using phthln. Calc. WIA in mg/100 g fat. 1 ml 0.05N Na ethylate = 13.5 mg WIA.

15.134 **Volatile Acids—Official**

Weigh 50 g sample into each of 2 centrifuge bottles and proceed as in **15.125**, beginning "Add 1N NaOH . . . " and continuing thru second washing with mixed ethers to remove fat. Then remove residual ethers from bottles by evapn on steam bath, transfer contents of both to single 200 ml vol. flask with H_2O, and add 1 ml H_2SO_4 (1+1) and 10 ml *10% $Na_2WO_4.2H_2O$ soln*. Dil. to mark, mix, and filter. Transfer 150 ml filtrate to distn flask and proceed with distn, chromatographic sepn, and detn as in **18.019–18.021**. Calc. to mg acids/100 g fat.

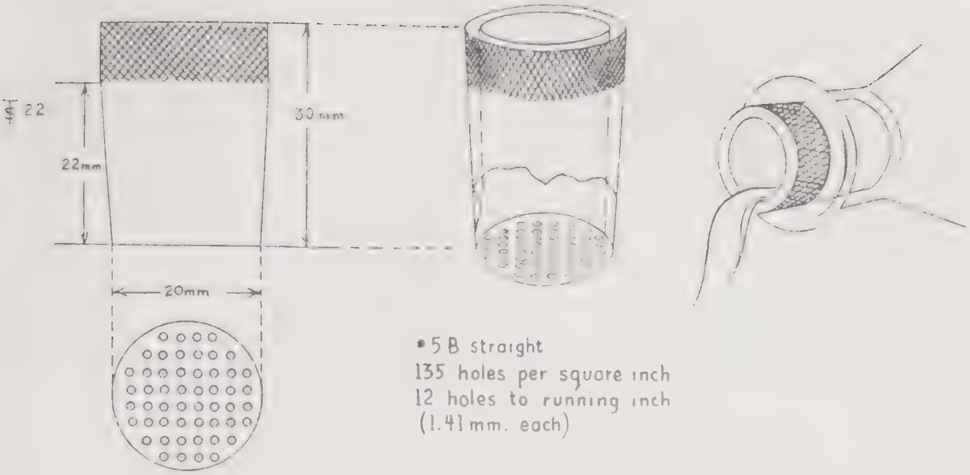

FIG. 29.—FILTER SIEVE

15.135 Preservatives Official See **15.041** and Chap. **27**

15.136 Microscopic Examination - Procedure

(**a**) Place on slide small portion of fresh unmelted sample taken from inside of the mass, add drop of pure olive oil, apply cover-glass with gentle pressure, and examine under 120–150× magnification for lard crystals, etc. Examine another portion of sample with polarized light and selenite plate without use of oil. Pure fresh butter shows neither crystals nor parti-colored field with selenite. Renovated butter or other fats melted and cooled and mixed with butter usually present crystals and variegated colors with selenite plate.

(**b**) For further microscopic study dissolve in test tube 3–4 ml of the fat in 15 ml ether. Close tube with loose plug of cotton wool and let stand 12–24 hr at 20–25°. When crystals form at bottom of tube, remove with pipet, glass rod, or tube; place on slide, cover, and examine under microscope. Crystals formed by later deposits may be examined in similar way. Compare with crystals obtained in same manner from samples of known purity.

15.137 Residual Phosphatase (*17*) **– Official**

See **15.056**. Take sample from beneath surface with clean knife or spatula and proceed as follows:

Step 1.—Weigh 1.0 g sample (preferably in duplicate) on piece of waxed paper ca 1″ square and insert paper with sample into tube. Similarly, weigh another sample and place in tube as control or blank.

Step 2. Heat *blank* ca 1 min. to 85 90° in beaker of boiling H_2O (covered so entire tube is heated to 85 90°), and cool to room temp. From this point treat blank and test alike.

Step 3.—Add 10.0 ml buffer substrate prepd as in **15.054**(**b**), except dissolve the $Na_2C_6H_5PO_4$ in 100 ml undild Ba borate-hydroxide buffer made from 18 g $Ba(OH)_2.8H_2O$ and 8 g H_3BO_3/L. Stopper tube and mix.

Step 4.—Immediately after adding substrate, incubate 1 hr in H_2O bath at 37–38°, mixing or shaking contents occasionally.

Step 5.—Heat in beaker of boiling H_2O nearly 1 min., heating to 85–90° (use thermometer in another tube of same size and shape contg same vol. of liquid), and cool to room temp. in vessel of cold H_2O.

Step 6.—Pipet in 1 ml 6.0 g/100 ml soln of $ZnSO_4.7H_2O$, and mix thoroly (pH of mixt. should be 9.0–9.1).

Step 7.—Filter (5 cm funnel, 9 cm Whatman No. 42 or No. 2 paper recommended), and collect 5.0 ml filtrate in tube, preferably graduated at 5.0 and 10.0 ml.

Steps 8–11.—Proceed as in **15.056**.

Step 12.—When using 1.0 g butter and adding 11.0 ml liquid, multiply value of reading by 1.1 to convert result to phenol equivs/0.5 g butter. (Values >2 equivs/0.5 g indicate underpasteurization.)

CHEESE

15.138 Collection of Sample -Procedure

When cheese can be cut, take narrow, wedge-shape segment reaching from outer edge to center. When not permissible to cut cheese, take sample with cheese trier. If only one plug can be obtained, take it perpendicular to surface of cheese at point ⅓ distance from edge to center and extending either entirely or half way thru. When possible draw 3 plugs, 1 from center, 1 near outer edge, and 1 midway between other two. Use ca ¾″ of rind portion of core to reseal hole.

Sample bulk containers of cottage and similar cheeses by stirring can thoroly for at least 5 min. with dairy stirrer (ca 5½″ perforated concave metal disk attached to ca 27″ metal rod as handle) so that all portions of container are reached. Remove portions from top surface with small spoon to fill pint jar and cover.

15.139 Preparation of Sample—Procedure

Cut wedge sample into strips and pass 3 times thru food chopper. Grind plugs in food chopper (preferable method), or cut or shred very finely and mix thoroly.

With cottage and similar cheeses, place 300–600 g sample at <15° in quart cup of high speed blender and blend for min. time (2–5 min.) required to obtain homogeneous mixt. Final temp. should be not >25°. This may require stopping blender frequently after channeling and spooning cheese back into blades until blending action starts. (Use of variable transformer in line to permit slow speed at first minimizes channeling when speed is increased later.)

Moisture

15.140 *Method I. (31)—Official*

Weigh 2–3 g prepd sample, **15.139,** into round, flat-bottom metal dish, not <5 cm diam. and provided with tight fit, slip-in cover. In case of soft cheese and process cheese of high moisture content, weigh 1–2 g and partially dry on steam bath. Place loosely covered dish on metal shelf (dish resting directly on shelf) in vac. oven, kept at temp. of boiling H_2O. Dry to constant wt (ca 4 hr) under pressure not >100 mm (4″) of Hg. During drying admit into oven slow current of air (ca 2 bubbles/sec.) dried by passing thru H_2SO_4. Stop vac. pump and carefully re-admit air into oven. Press cover tightly into dish, remove dish from oven, cool, and weigh. Express loss in wt as moisture.

15.141 *Method II. (Rapid Screening Method) (32)—First Action*

Weigh 2–3 g prepd sample into moisture dish with tight-fitting cover. Partially dry on steam bath with lid removed and then insert in forced draft oven that has come to equilibrium at 130±1°. Dry 1.25 hr (with cover entirely off), cover tightly, remove from oven, cool, and weigh.

15.142 Ash *(33)*—**Official**

Weigh 3–5 g prepd sample, **15.139,** into Pt dish, place on steam bath, and dry ca 1 hr. (If cheese is high in fat, place small amount of absorbent cotton in dish.) Ignite cautiously to avoid spattering and remove burner while fat is burning. When flame ceases, complete ignition in muffle at temp. not >550°.

15.143 Total Chlorides *(33)*—**Official**

Weigh ca 3 g prepd sample, **15.139,** into 200 ml erlenmeyer and add 25 ml 0.1*N* $AgNO_3$ which is more than enough to combine with all the Cl. Add 10 ml halogen-free HNO_3 and 50 ml H_2O, and boil. As soln boils, add ca 15 ml 5% $KMnO_4$ soln in 5 ml portions. (Soln becomes yellowish and clear.) Cool, filter into 200 ml vol. flask, washing paper thoroly with H_2O at ca 20°, and dil. to vol. Titr. excess $AgNO_3$ in 100 ml clear soln with 0.1*N* KSCN, using 2 ml satd soln of Fe alum as indicator. Det. blank on reagents used, following same procedure, except to add sugar to destroy excess $KMnO_4$. Calc. Cl found to NaCl.

15.144 Nitrogen—Official

Det. N in weighed portion (ca 2 g) prepd sample, **15.139,** as in **2.036.** % N×6.38=% "protein."

15.145 Acidity—Official

To 10 g prepd sample, **15.139,** add H_2O at 40° to vol. of 105 ml, shake vigorously, and filter. Titr. 25 ml portion filtrate, representing 2.5 g sample, with std NaOH, preferably 0.1*N*, using phthln. Express result as lactic acid. 1 ml 0.1*N* NaOH=0.0090 g lactic acid. Results may also be expressed as ml 0.1*N* NaOH/100 g.

15.146 Coloring Matters—First Action

Ext. 25–50 g prepd sample, **15.139,** with ether, remove ether by evapn, and proceed as in Chap. **35.**

15.147 Fat *(34)*—**Official**

In small, tall beaker rub 1 g prepd sample, **15.139,** to smooth liquid with 9 ml H_2O and 1 ml NH_4OH. Digest mixt. at low heat until casein is well softened; neutralize with HCl, using litmus as indicator; and add 10 ml more HCl. Add few glass beads or other inert material, previously digested with HCl, to prevent bumping, and boil gently 5 min., keeping beaker covered with watch glass. Cool soln, transfer to Mojonnier fat-extn flask or Röhrig tube, rinse beaker with 25 ml ether, and transfer ether rinsings to flask or tube, shaking thoroly. Add 25 ml petr. ether (b. p. <65°), shake thoroly, and proceed as in **15.029,** beginning "Centrifuge Mojonnier flask . . . "

15.148 Examination of Fat—Official

(a) *Alkaline extraction.*—In large, wide-mouth flask, treat ca 300 g sample, cut to ca pea-size, with 700 ml 5% KOH soln at 20°, shaking vigorously to dissolve casein. (In 5–10 min., casein dissolves, and fat rises to surface in lumps.) Col-

lect lumps of fat into as large mass as possible by shaking gently. Pour cold H_2O into flask until fat is driven up into neck, and remove by suitable means. Wash fat thus obtained with just enough H_2O to remove residual alkali. Fat is not perceptibly attacked by alkali in this treatment, is practically all sepd in short time, and is then easily prepd for chemical analysis by filtering and drying as in **15.119.** Examine fat as in Chap. **26.**

(b) *Acid extraction.*—Pass cheese thru grinding machine, transfer to large flask, and cover with warm H_2O, using 1 ml/g cheese. Shake thoroly and add H_2SO_4 slowly and in small quantities, shaking after each addn of acid. (Vol. H_2SO_4 should equal vol. H_2O used.) Remove fat, which seps after standing few min., in separator; wash free from sulfate, filter, and dry as in **15.119.** Examine fat as in Chap. **26.**

Tartaric Acid (*35*)

15.149 *Qualitative Test—Procedure*

To 5 g ground cheese, **15.139,** add 40 ml H_2O at ca 50° and shake until cheese is thoroly broken up. Add 3 ml 1% H_2SO_4 and shake vigorously. Add 2 ml *20% phosphotungstic acid soln* and again shake vigorously. Let stand 5 min. and filter. To 25 ml filtrate add enough satd $Ba(OH)_2$ soln to make alk. and 25 ml alcohol, shake vigorously, and let settle. Filter thru büchner, using light suction, and wash residue on filter several times with H_2O. Transfer portion of paste to small evapg dish and dry on steam bath. Add few ml H_2SO_4 and few crystals of *resorcin,* and heat slowly. If tartaric acid is present, rose-red color is produced that is slowly discharged on diln with H_2O.

Quantitative Method (35)—Official

15.150 REAGENTS

(a) *Potassium chloride wash soln.*—Dissolve 15 g KCl in 100 ml H_2O and add 20 ml alcohol.

(b) *Tartaric acid soln.*—Dissolve 1.5 g pure tartaric acid in previously boiled and cooled H_2O and dil. to 100 ml at 20°. Titr. with $0.1N$ NaOH to det. tartaric acid in 10 ml soln.

(c) *Hydrochloric acid soln.*—2%. Dil. 47 ml HCl to 1 L with H_2O.

15.151 DETERMINATION

Weigh 25 g prepd sample, **15.139,** into 500 ml wide-mouth bottle and add, 25 ml at time, 100 ml H_2O at 50–60°, shaking vigorously after each addn. Continue shaking until cheese is thoroly broken up. Add 25 ml *2% $Na_2C_2O_4$ soln* and shake vigorously 1 min. Add 100 ml of the 2% HCl, 25 ml at time, shaking vigorously after each addn. Add 50 g powd. KCl and shake 5 min. To avoid churning, keep mixt. warm (ca 50°) during shaking. Transfer mixt., with aid of H_2O, to 300 ml vol. flask, cool to 20°, and dil. to mark with H_2O. Mix thoroly; let stand 10 min., with occasional shaking; and filter thru dry folded paper, discarding first few ml filtrate. Disregard any opalescence and transfer 200 ml filtrate to 250 ml vol. flask. Neutralize with $1N$ NaOH, using phthln, and add 5.2 ml in excess. Dil. to mark with H_2O, mix thoroly, let stand few min., and filter thru dry folded paper, discarding first few ml filtrate.

To 100 ml filtrate in 250 ml beaker add, with constant stirring, 10 ml of the tartaric acid soln, 2 ml HOAc, and 23 ml alcohol. Cool in ice bath, stir vigorously until cream of tartar begins to crystallize, and let stand in refrigerator overnight. Prep. Caldwell crucible with pad of asbestos ca 10 mm thick. Decant most of liquid thru this filter, wash ppt into crucible with the KCl wash soln, and wash beaker and ppt 3 times, using total quantity of 20–30 ml of the wash soln. Place asbestos and ppt in beaker in which pptn was made and wash crucible with ca 50 ml hot H_2O. Heat soln to boiling and titr. while hot with $0.1N$ NaOH, using phthln. Calc. % tartaric acid in cheese by formula:

$X = 14.26[0.015(B+1.5) - A]$, where A = g tartaric acid in 10 ml of the tartaric acid reagent; and B = ml $0.1N$ NaOH required for titrn.

In factor 14.26, concn caused by insol. solids of cheese of av. composition is taken into consideration.

Citric Acid (*36*)

15.152 *Qualitative Test—Procedure*

To 10 g prepd sample, **15.139,** add 20 ml H_2O at ca 50° and shake vigorously until cheese is thoroly broken up. Add 20 ml H_2SO_4 (1+1) and 2 ml *20% phosphotungstic acid soln,* and shake vigorously. Let stand 5 min. and filter. To 20 ml filtrate add 10 ml Br-H_2O and 5 ml KBr soln (15 g in 40 ml H_2O), and proceed with oxidation as in **15.153.** Add enough $FeSO_4$ soln (**15.153,** par. 3) to dissolve pptd MnO_2. If citric acid is present, heavy white ppt is formed which settles rapidly.

15.153 *Quantitative Method—Official*

Prep. suspension as in **15.151** thru addn of $Na_2C_2O_4$ soln. Shake vigorously 1 min. and add 100 ml 1% H_2SO_4, 25 ml at time, shaking vigorously after each addn. Add 3 ml *20% phosphotungstic acid soln* and shake; then add 25 g powd. anhyd. Na_2SO_4, and shake 5 min. To avoid churning, keep mixt. warm (ca 50°) during shaking. Transfer mixt. with aid of warm H_2O to 300 ml vol. flask, cool to 20°, and dil. to mark with H_2O. Mix thoroly, shake occasionally during 10 min., and filter thru dry folded paper, discarding first few ml filtrate.

Heat 200 ml filtrate to boiling and while still hot add 20 ml H_2SO_4 (1+1) and 2 ml of the phosphotungstic acid soln. Mix and let stand 15 min. With aid of H_2O transfer mixt. to 250 ml vol. flask, cool to 20°, dil. to mark with H_2O, and filter thru dry folded paper.

Transfer 100 ml clear filtrate to 500 ml erlenmeyer (ca 0.3 g washed and dried asbestos may be added). Add 10 ml freshly prepd satd Br-H_2O and 5 ml KBr soln (5 g KBr in 40 ml H_2O), mix thoroly, and heat to 48–50°. Hold at this temp. 5 min., add 25 ml 5% $KMnO_4$ soln, shake, and let stand ca 5 min. Cool flask and contents to ca 8°, add 40 ml cold *$FeSO_4$ soln* (20 g $FeSO_4.7H_2O$ in 100 ml H_2O and 1 ml H_2SO_4), shake continuously 5 min., and let mixt. stand overnight in refrigerator.

Decant supernatant thru gooch, measure vol. filtrate (*a*), and wash ppt from erlenmeyer into crucible with this filtrate. Wash ppt with three 20 ml portions ice-cold H_2SO_4 (1+100), sucking dry after each addn, and finally wash with three 20 ml portions ice-cold H_2O. Dry ppt to constant wt over H_2SO_4 in vac. desiccator, protecting ppt from strong light or, to save time, dry in current of air passed thru H_2SO_4, and weigh.

Remove pentabromacetone by extg first with three 20 ml portions alcohol and then with three 20 ml portions ether. Dry and weigh crucible. To wt pentabromacetone add 0.004 g/100 ml filtrate (*a*) to compensate for solubility of pentabromacetone and multiply result by 6.06 to obtain % anhyd. citric acid in cheese. (In this factor concn caused by insol. solids in 25 g cheese is taken into consideration. It is assumed that solids of cheese are almost insol. under conditions maintained and that av. process cheese contains ca 60% solids. No allowance is made for variation in salt or moisture content or for variation in specific vol. of solids, as such variations do not appreciably affect results.)

15.154 Lactose in Process Cheese (*37*)—Official

Prep. suspension as in **15.151** thru addn of $Na_2C_2O_4$ soln. Shake vigorously 1 min.; add 25 g powd. Na_2SO_4 and shake 2 min.; add 10 ml H_2SO_4 (1+1) and shake; then add 25 ml *20% phosphotungstic acid soln* and shake vigorously. Transfer contents of bottle to 500 ml vol. flask, cool at once to 20°, and dil. to mark with H_2O. Mix thoroly, let stand 10 min., and filter thru dry folded paper. Transfer 150 ml filtrate to each of two 250 ml vol. flasks, add 10% NaOH soln to one flask until mixt. is alk. to litmus, and then add 5 g solid KCl and mix thoroly. Cool to 20° and dil. to mark with H_2O. Mix well, let stand 10 min., and filter thru dry folded paper.

Det. lactose in 50 ml aliquot as in **29.039.** Treat the 150 ml in second flask as in **29.026(c)**, using 10 ml HCl, etc. Add 10% NaOH soln until alk. to litmus, and add 5 g solid KCl. Mix thoroly, cool to 20°, and dil. to mark with H_2O. Let stand 10 min. Filter if necessary thru dry paper. Det. lactose in 50 ml aliquot as before. Agreement between quantities of Cu_2O reduced before and after inversion establishes absence of sucrose.

Since insol. material of cheese and phosphotungstic acid ppt occupy some space in flask as originally made up, it is necessary to correct for this vol. From av. composition of cheese, vol. of ppt was calcd to be 14 ml. To obtain true quantity of lactose present, multiply all results by factor 0.97.

Gums in Soft Curd Cheese (*38*)—Official

(Not applicable to detection of alginates)

15.155 REAGENTS

(**a**) *Benedict soln* (*qualitative*).—Dissolve 17.3 g Na citrate and 10 g anhyd. Na_2CO_3 in ca 80 ml hot H_2O; dissolve 1.73 g $CuSO_4.5H_2O$ in 10 ml H_2O. Filter the alk. citrate soln, add the $CuSO_4$ soln slowly with constant stirring, and dil. with H_2O to 100 ml.

(**b**) *Trichloroacetic acid soln.*—50%. Prep. just before use from non-hydrolyzed reagent.

(**c**) *Dilute trichloroacetic acid soln.*—10%. Note warning in (**b**).

15.156 PREPARATION OF SAMPLE

Weigh 100 g cheese into 250 ml centrifuge bottle. Add hot H_2O to total vol. of 170 ml, heat in hot H_2O bath 30 min., and cool to room temp Add 50 ml petr. ether, shake, and centrifuge. Remove petr. ether layer by decantation or by use of a blow-off siphon, **15.125,** Notes. Repeat extn with petr. ether at least twice. (Small amount of fat remaining will do no harm.)

Warm bottle in hot H_2O bath to remove residual petr. ether from cheese. Centrifuge, if necessary, to break any foam. Make vol. to ca 190 ml with H_2O and add 3.5 ml NH_4OH, few drops at time, while stirring. Keep in hot H_2O bath and stir until all curd dissolves. If curd fails to dissolve completely, add few more drops NH_4OH, stir, and macerate to dissolve. Add HOAc, few drops at time, with shaking, until pH is ca 4.75 (nitrazine test paper or pH meter). Use care in approaching pH point because isoelectric point for casein is ca pH 4.73. (If acid is added very slowly with constant shaking and centrifuge bottle is kept hot marked sepn of casein and liquid will be noted at this point.) Stopper bottle, shake thoroly, and let stand overnight in the hot H_2O bath as H_2O cools. Check pH and centrifuge at 1200 rpm 10 min. Decant supernatant into 250 ml beaker with 40 ml graduation mark. Do not wash ppt.

15.157 SEPARATION OF GUM

Evap. decanted liquid on steam bath to 40 ml mark of beaker. Remove beaker from bath and cool to room temp. Disregard ppt formed during concn and add 10 ml of the 50% trichloroacetic acid soln (note warning under REAGENTS). Replace on steam bath for at least 15 min. to coagulate protein. Remove beaker from steam bath, cool, transfer to 250 ml centrifuge bottle with 5 ml of the dil. trichloroacetic acid soln, and centrifuge at 1200 rpm 10 min. Decant supernatant into another 250 ml centrifuge bottle and add alcohol with stirring until bottle is full. (Vol. before addn of alcohol should be not >50 ml and ca 4 vols alcohol should be added.) Let mixt. stand at least 1 hr to coagulate gums. Centrifuge at 1800 rpm, decant, and discard liquid.

Add ca 50 ml 70% alcohol to residue in bottle, stopper, and shake to break up material thoroly. Wash down stopper and sides of bottle with little 70% alcohol, centrifuge at 1800 rpm, decant, and drain. Add 40 ml hot H_2O to bottle and shake well to dissolve gum and disperse insol. material. Add 10 ml of the 50% trichloroacetic acid to bottle and heat on steam bath 15 min. to coagulate any protein left after first treatment.

Remove bottle, cool, and centrifuge at 1200 rpm 10 min. Decant supernatant into another 250 ml Pyrex centrifuge bottle, and fill bottle with alcohol while stirring contents. Add 0.5 ml 5% *$KAl(SO_4)_2$ soln*. Shake, and let stand at least 1 hr. Centrifuge at 1800 rpm and decant. Add 50 ml 70% alcohol, shake to disperse material, and centrifuge at 1800 rpm. Decant supernatant and drain. Add 40 ml hot H_2O and shake well to dissolve gum. Transfer to 50 ml conical heavy-duty centrifuge tube, keeping vol. to 40 ml. Centrifuge at 1200 rpm 10 min. to remove any undissolved material, and decant supernatant back into 250 ml centrifuge bottle. Reppt in bottle by filling with alcohol plus 1 drop HOAc. To insure pptn of gum tragacanth and karaya, add 0.5 ml 5% $KAl(SO_4)_2$ soln.

15.158 DETECTION OF GUM

Let ppt coagulate as before, centrifuge, and decant liquid. If ppt is small and will not remain on bottom of 250 ml centrifuge bottle, centrifuge, portion at time, the alcohol and pptd gum at 1500 rpm 15 min. in 50 ml conical heavy-duty centrifuge tube, until all contents of 250 ml bottle are transferred to 50 ml tube. After decanting supernatant from last portion centrifuged, add 40 ml 70% alcohol to tube (or bottle if tube is not used), and shake until ppt is dispersed; centrifuge, decant, and drain.

Add to residue in tube or bottle 10 ml hot H_2O, shake, and transfer to 50 ml beaker. Rinse tube or bottle with 10 ml hot H_2O and add rinse to beaker. Warm on elec. hot plate to dissolve gum and evap. to 10 ml. Add 2 ml HCl, cover beaker with watch glass, and boil gently 5 min. Cool, transfer to 10 ml graduated cylinder, adjust to 10 ml with H_2O, and mix. Place 1 ml aliquot in 30 ml beaker and neutralize with 10% NaOH soln, using litmus paper as indicator. Remove litmus paper, add 5 ml Benedict's soln, boil vigorously 2 min., and let cool spontaneously. Voluminous ppt appearing on cooling, which may be yellow, orange, or red, caused by reducing sugars formed by hydrolysis of the gums, indicates presence of gums.

Gelatin in Cottage Cheese

15.159 *Qualitative Test—Official*

Mix thoroly 5 g sample with 10 ml H_2O at 50–60° and add 5 ml $Hg(NO_3)_2$ soln, **15.040.** Shake, let stand 5 min., and filter thru medium-fast retentive paper. To filtrate add 5 ml more of the $Hg(NO_3)_2$ soln and test as in **15.040,** using filtrate so obtained. *See also* NOTE in **15.040.**

Residual Phosphatase (*17*)—Official

15.160 REAGENTS

(**a**) *Buffers:*

(1) *25-11 Barium borate-hydroxide buffer.—See* **15.054**(**a**)(1).

(2) *26-11, 27-11, 28-11, and 29-11 Barium borate-hydroxide buffers.*—Prep. as in **15.054**(**a**)(1), except use 26.0, 27.0, 28.0, or 29.0 g $Ba(OH)_2.8H_2O$, resp., instead of 25.0 g.

(**b**) *Buffer substrates:*

Dissolve 0.10 g phenol-free cryst. $Na_2C_6H_5PO_4$ in 100 ml appropriate buffer, (**a**), specified in **15.163.** *See* **15.054**(**b**)(1) for purification of phenol-free substrate.

(**c**) *Protein precipitants:*

(1) *6.0–0.1 Precipitant.*—Dissolve 6.0 g $ZnSO_4.7H_2O$ and 0.1 g $CuSO_4.5H_2O$ in H_2O and dil. to 100 ml.

(2) 6.0 *Precipitant.*—Dissolve 6.0 g $ZnSO_4.7H_2O$ in H_2O and dil. to 100 ml.

For other reagents, *see* **15.054.**

15.161 SAMPLING

(**a**) *Hard cheese.*—Take sample from interior with *clean* Roquefort trier, place in small tube, stopper, and keep in refrigerator.

(**b**) *Soft and semi-soft ripened cheese.*—Harden cheese by chilling in freezing compartment of refrigerator. Taking special precautions to avoid contaminating sample with phosphatase that may be present on surface, sample by either of following methods:

(1) Cut portion from end of loaf or side of cheese, extending in at least 2″ if possible, to point somewhat beyond center in case of small

cheese. Cut slit $\frac{1}{4}$–$\frac{1}{2}''$ deep at least half way around portion and midway between top and bottom. Break portion into 2 parts, pulling apart so that break occurs on line with slit and taking care not to contaminate freshly exposed broken surface. Remove sample from freshly exposed surface at or near center of cheese.

(2) Remove surface of area to be sampled (*e.g.*, end and adjacent sides), with clean knife or spatula, to depth of $\frac{1}{4}''$. Clean instrument and hands with hot H_2O and phenol-free soap, and wipe dry. Remove freshly exposed surface to same or greater depth, and repeat cleaning. Then take sample from center of freshly exposed area, preferably at or near center of cheese if cheese is small.

(**c**) *Process cheese and cheese spreads.*—Take sample from beneath surface with clean knife or spatula.

If preservative is necessary, put 1–3 ml $CHCl_3$ in container, cover with plug of cotton, insert sample, and stopper tightly. Label "*Poison, preservative added.*"

15.162 DETERMINATION

(*See* **15.056.**) Different kinds of cheese and cheeses of different ages have different buffering capacities and therefore require different concns of reagents. Modifications of the Ba buffer needed to produce optimal pH conditions during incubation (9.85–10.20) and of precipitant to yield uniformly clear filtrates and minimize interference during development of color under optimal pH conditions (9.3–9.4) are specified in **15.163.**

Proceed as follows:

Step 1.—Weigh, on clean balance pan or watch glass, 0.50 g sample (preferably in duplicate) and place in culture tube 16 or 18×150 mm. Similarly weigh another sample and place in tube as control or blank. If cheese is sticky, weigh sample on piece of wax paper ca $1\times1''$ and insert paper with sample into tube. Macerate blank and test samples with glass rod ca 8×180 mm.

Step 2.—Add to *blank* 1.0 ml appropriate Ba buffer, **15.163** (without substrate), macerate with rod, leave rod in tube, and heat ca 1 min. to 85–90° in beaker of boiling H_2O (covered, to insure that entire tube will be heated to 85–90°); cool to room temp., and again macerate with rod.

Step 3.—Add *to each test sample* 1.0 ml appropriate Ba buffer substrate, **15.160** (**b**), and macerate.

From this point, treat blank and test alike.

Add 9.0 ml more Ba buffer substrate (total 10.0 ml), and mix. (Rod may be left in tube during incubation. If it is removed at this point, wrap piece of ca $1\times1''$ filter paper tightly around it and wipe it clean by rotating while withdrawing from tube. Insert paper with adhering fat in tube.) Stopper tube.

For precise quant. results on unknown samples, adjust pH to 10.0–10.05 by dropwise addn of $1N$ or $0.5N$ Na_2CO_3 or HCl.

Step 4.—Immediately after performing above operations, incubate in H_2O bath 1 hr at 37–38°, mixing or shaking contents occasionally.

Step 5.—Heat in beaker of boiling H_2O nearly 1 min. (temp. of contents of tube ca 85° as detd by thermometer in another tube of same size and shape contg same vol. liquid), and cool to room temp. in vessel of cold H_2O.

Step 6.—Pipet in 1.0 ml appropriate protein precipitant, **15.163,** and mix thoroly (pH of mixt., 9.0–9.1).

Steps 7–11.—Proceed as in **15.056.**

Step 12.—When using 0.5 g solid sample and adding total of 11.0 ml liquid, multiply value of reading by 1.1 to convert to units of color or phenol equivs/0.25 g cheese. (If desired, multiply by 4.4 to convert result to phenol equivs/g.) Evaluate result by comparing with criteria of pasteurization in **15.163.**

NOTES: With some cheese samples of unknown history, slight deviations from optimal pH range may occur, but such deviations do not materially affect results. For example, pH values as low as 9.6 or as high as 10.35 during incubation have been found to result in av. decrease of not $>20\%$ in quantity of phenol liberated. Use of 25-11 buffer substrate with samples for which 27-11 buffer substrate is specified yields pH values not <9.8.

Trace of cloudiness in filtrate, following use of prescribed precipitant, indicates concn of $Ba(OH)_2$ in buffer was insufficient (*i.e.*, buffer substrate was insufficiently alk.). For example, the 25-11 buffer, for use with unripened cheese, may yield cloudy filtrate if used with ripened cheese. Increasing concn of $ZnSO_4$ in precipitant also eliminates turbidity of filtrate.

In testing cheese of unknown history or age, information as to % solids, especially nonfat solids, is useful as indication of correct buffer to use; cheese with relatively high % of nonfat solids generally requires use of relatively concd buffer to adjust pH of mixt. correctly. Av. buffer within cheese group (generally 26-11) is usually satisfactory for cheese of uncertain age.

Cottage cheese curd is heated in presence of considerable acid during manufacture, and therefore its phosphatase values are comparatively low. To increase sensitivity of test on cottage cheese, apply following modifications: Use 1.0 g sample, 27-11 buffer substrate, 2 hr incubation, 6.0–0.1 precipitant, and pasteurization criterion of 2 units/0.5 g.

To test for presence of microbial phosphatase, *e.g.*, in surface ripened cheeses and their processed products, (**a**) indicated by blue color in blank of *Step 2*, repeat detn, adding 1 ml of the Ba buffer (without substrate) to blank and heating 5 min. in boiling H_2O in covered beaker. If blank so treated is negative, blue color in original blank was due to microbial phosphatase. (**b**) In suspected instances in absence of blue color in blank of *Step 2*, heat sample itself 5 min. at 70° to completely destroy milk phosphatase and then run test. If positive, microbial phosphatase is present.

See also NOTES under **15.056.**

15.163 *Phosphatase test modifications for different kinds of cheese and cheese of different ages*

KIND OF CHEESE	AGE OR EXTENT OF CURING; OTHER DETAILS	BUFFER FOR OPT. PH[a] (9.85–10.20)	PRECIPITANT	CRITERION, EXPERIMENTAL, PHENOL EQUIVALENT[b]
				mmg/0.25 g
Cheddar, granular, stirred curd, hard cheese	<1 wk	25-11	6.0–0.1[c]	3
	1–6 wk	25-11	6.0[d]	3
	1.5–4 mo.	26-11	6.0[d]	3
	>4 mo.	27-11	6.0[d]	3
Washed curd, soaked curd, colby	<1 wk	25-11	6.0–0.1[c]	3
	1–8 wk	25-11	6.0[d]	3
	>2 mo.	26-11	6.0[d]	3
Swiss, gruyère	<1 wk	25-11	6.0–0.1[c]	3
	1–4 wk	25-11	6.0[d]	3
	1–3 mo.	26-11	6.0[d]	3
	>3 mo.	27-11	6.0[d]	3
Brick, muenster	<1 wk	25-11	6.0–0.1[c]	3
	1–4 wk	25-11	6.0[d]	3
	1–2 mo.	25-11	6.0[d]	3
	>2 mo.	26-11	6.0[d]	3
Edam, gouda	<1 wk	25-11	6.0–0.1[c]	3
	1–8 wk	25-11	6.0[d]	3
	2–4 mo.	26-11	6.0[d]	3
	>4 mo.	27-11	6.0[d]	3
Blue mold, blue, gorgonzola	<1 wk	25-11	6.0–0.1[c]	3
	1–4 wk	26-11	6.0[d]	3
	1–4.5 mo.	27-11	6.0[d]	3
	>4.5 mo.	28-11	6.0[d]	3
Camembert, limburger	<1 wk	25-11	6.0–0.1[c]	4
	1–4 wk	25-11	6.0[d]	4
	1–2 mo.	26-11	6.0[d]	4
	>2 mo.	27-11	6.0[d]	4
Monterey	<1 wk	25-11	6.0–0.1[c]	3
	1–8 wk	25-11	6.0[d]	3
	>2 mo.	26-11	6.0[d]	3
High moisture Jack	<1 wk	25-11	6.0–0.1[c]	3
	1–10 wk	25-11	6.0[d]	3
	>2.5 mo.	26-11	6.0[d]	3
Provolone, pasta filata	<1 wk	25-11	6.0–0.1[c]	3
	1–4 wk	25-11	6.0[d]	3
	1–3 mo.	26-11	6.0[d]	3
	>3 mo.	27-11	6.0[d]	3
Parmesan, reggiano, monte, nodena romano, asiago old	<1 wk	25-11	6.0–0.1[c]	3
	1–8 wk	26-11	6.0[d]	3
	2–6 mo.	27-11	6.0[d]	3
	6–12 mo.	28-11	6.0[d]	3
	>1 yr	29-11	6.0[d]	3
Asiago, fresh	Same as Cheddar			
Asiago, medium	<1 wk	25-11	6.0–0.1[c]	3
	1–4 wk	25-11	6.0[d]	3
	1–3 mo.	26-11	6.0[d]	3
	>3 mo.	27-11	6.0[d]	3

[a] $Ba(OH)_2.8H_2O$ and H_3BO_3, resp., g/L.
[b] Higher values indicate underpasteurization.
[c] $ZnSO_4.7H_2O$ and $CuSO_4.5H_2O$, resp., g/100 ml.
[d] $ZnSO_4.7H_2O$ g/100 ml.

15.163 *Phosphatase test modifications for different kinds of cheese and cheese of different ages*—Continued

KIND OF CHEESE	AGE OR EXTENT OF CURING; OTHER DETAILS	BUFFER FOR OPT. PH[a] (9.85–10.20)	PRECIPITANT	CRITERION, EXPERIMENTAL, PHENOL EQUIVALENT
				mmg/0.25 g
Cottage,[e] cook cheese, koch kaese	Dry	25-11	6.0–0.1[c]	1
	Moist	25-11 (8+2)[f]	4.5–0.1[c]	1
Cream cheese		25-11 (7+3)	4.5–0.1[c]	3
Semi-soft cheese	<1 wk	25-11	6.0–0.1[c]	3
	1–4 wk	25-11	6.0[d]	3
	>1 mo.	26-11	6.0[d]	3
Soft ripened cheese	<1 wk	25-11	6.0–0.1[c]	4
	1–4 wk	25-11	6.0[d]	4
	>1 mo.	26-11	6.0[d]	4
Nokkelost, kuminost, sage cheese	<1 wk	25-11	6.0–0.1[c]	3
	1–6 wk	25-11	6.0[d]	3
	1.5–4 mo.	26-11	6.0[d]	3
	>4 mo.	27-11	6.0[d]	3
Past. proc.; ditto, pimiento; ditto, with fruits, meats, etc.	Soft, mild	25-11	6.0[d]	3
	Med. firm	26-11	6.0[d]	3
	Firm, sharp (incl. swiss, gruyère)	27-11	6.0[d]	3
Past. proc. cheese foods; ditto, with fruits, meats, etc.	Same as past. proc.			
Past. proc. cheese spreads; ditto, with fruits, meats, etc.	Soft, high moisture, incl. cream spreads	25-11	6.0[d]	3
	Less soft, incl. blue	26-11	6.0[d]	3
Cold pack, club; cold pack cheese foods; ditto, with fruits, meats, etc.	Mild-med. flavored, soft	26-11	6.0[d]	3
	Sharp, firm	27-11	6.0[d]	3

[e] See also more sensitive modification in text, alternative.
[f] 8 parts 25-11 buffer +2 parts of H_2O.

ICE CREAM AND FROZEN DESSERTS

Weight per Unit Volume of Packaged Ice Cream (39)—Official

15.164 APPARATUS

(a) *Overflow can.*—Fig. 30. No. 10, or 1 gallon can with overflow spout of $\frac{1}{8}$–$\frac{3}{16}$″ i. d. metal tubing soldered to opening in side of can ca 1″ from bottom and bent upward and extending parallel to sides. Tube should be bent over at upper end to form spout ca 1½″ below top of can. Upper edge of opening of spout should be above and lower edge below highest point of interior surface of top bend, *A*. Iron bar, slightly longer than diam. of can, equipped with "bridge" of tinned metal, may be used to submerge sample in kerosene of known density at 20/4° and cooled to 5–10° (icebox temp.) before use. "Bridge" should extend ½″ below level of *A*.

(b) *Balance.*—Capacity 1 kg, sensitive to 1 g.

(c) *Cylinders or beakers.*—500 to 1000 ml, graduated.

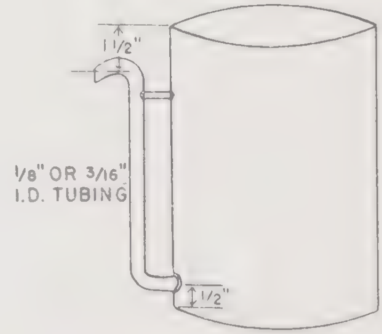

FIG. 30.—OVERFLOW CAN

15.165 DETERMINATION

Obtain packaged samples (pints preferred) from freezing compartment or cold room and immediately place in insulated container with Dry Ice for transporation to laboratory. Surround package with slabs or pieces of Dry Ice until frozen solid.

Place overflow can on level table so that overflow discharges into sink. Fill can with the cooled

kerosene until it overflows thru spout. When overflow ceases, place tared 500 ml graduated cylinder (or beaker) under spout.

Remove frozen brick from Dry Ice, quickly remove from carton, and weigh to accuracy of 1–2 g. Designate this wt W. Slowly immerse brick in the kerosene, finally submerging it completely by holding it under surface with small spatula, or "bridge" described in **15.164**(a), until overflow ceases. Weigh displaced kerosene to accuracy of 1–2 g, and subtract tare wt cylinder or beaker to ascertain net wt kerosene displaced. Divide net wt kerosene by its sp. gr. and designate resultant vol. V.

$$\text{Wt/unit vol. (as lb/gal.)} = W \times 8.345/V.$$

If products are so packed that they are difficult to remove from carton, det. gross wt carton and and contents, then open ends or sides of carton enough to avoid formation of entrapped air bubbles, and submerge entire carton and contents in the kerosene as directed. After overflow ceases and displaced kerosene has been weighed, remove contents from carton, dry empty carton, and weigh. Transfer the kerosene to 100 ml or 200 ml graduated cylinder, filling to half-way mark, and record vol. Roll up dried carton so that it will slip into cylinder, avoiding entrapment of air. Push carton into cylinder until it is completely immersed in the kerosene. Increase in vol. is vol. occupied by carton. Correct for wt and vol. of carton in formula given above and calc. unit wt in lb/gallon.

Graduated cylinder may be used instead of beaker to catch overflow. Vol. reading may be used as check against calcd vol. of the kerosene. Vol. as calcd from wt is more nearly accurate.

15.166 Preparation of Sample (*40*)—Procedure

(**a**) *Plain products.*—Let sample soften at room temp. Because melted fat tends to sep. and rise to surface, it is not advisable to soften sample by heating on H_2O bath or over flame. Mix thoroly by stirring with spoon or egg beater, or by pouring back and forth between beakers.

(**b**) *Frozen desserts containing insoluble particles.*—Use malted milk mixer capable of comminuting product to fine, uniform pulp.

Use enough sample (4–8 oz) to fill cup of mixer $\frac{1}{4}$–$\frac{1}{3}$ full. Melt at room temp., or in incubator set at 37°, in closed container (Mason jar is suitable), transfer entire sample to mixer cup, and mix until insol. particles are finely divided (2–5 min. for fruit ice creams, and up to 7 min. for nut and certain candy ice creams). Transfer mixed sample to suitable container for convenience in weighing.

15.167 Total Solids (*41*)—Official

Into round, flat-bottom dish not <5 cm diam., weigh quickly 1–2 g sample. (Sample may be weighed by means of short, bent, 2 ml measuring pipet.) Heat on steam bath 30 min. and then in air oven 3.5 hr at 100°. Cool in desiccator and weigh quickly to avoid absorption of moisture.

15.168 Nitrogen—Official

Proceed as in **2.036**, using 4–5 g sample. % N × 6.38 = % "protein."

Fat

15.169 *Roese-Gottlieb Method* (*42*)—*Official*

Weigh accurately 4–5 g thoroly mixed sample directly into Mojonnier fat-extn flask (or Röhrig tube or similar app.), using free-flowing pipet; dil. with H_2O to ca 10 ml, working charge into lower chamber and mixing by shaking. Add 2 ml NH_4OH, mix thoroly, and heat in H_2O bath 20 min. at 60° with occasional shaking. Cool, and proceed as in **15.029**, beginning "Add 10 ml alcohol and mix well."

15.170 Separation of Fat from Ice Cream—Official

Melt sample and screen out any large pieces of fruit, nuts, etc. on No. 20 sieve. Place 300 ml melted sample in 1 L separator, add 100 ml H_2O and 50 ml NH_4OH, and shake well. Add 200 ml alcohol and shake 1 min. Add 200 ml ether and shake 1 min. Add 200 ml petr. ether and shake 1 min. Let stand until emulsion breaks, and drain and discard lower layer. Add 25 g anhyd. Na_2SO_4, shake, and decant thru rapid folded paper. Evap. ether and alcohol, and dry fat at 55° overnight. Examine fat as in Chap. **26**.

15.171 Lactic Acid—Official—*See* 15.008–15.013

Gums

Infrared Method (*43*)—*First Action*

(Guar flour gum and locust bean gum cannot be distinguished by this method. Karaya gum cannot be identified when isolated by this method.)

15.172 REAGENTS

(**a**) *Dioxane.*—Technical grade is satisfactory. (*Caution*: Vapors are obnoxious and harmful.)

(**b**) *Trichloroacetic acid soln.*—50%. Freshly prepd as needed from non-hydrolyzed reagent.

(**c**) *Organo-silicone compound.*—To prep. nonwettable surface. Desicote ® (Scientific Instruments Div., Beckman Instruments, Inc., Fullerton, Calif.) has been found satisfactory.

15.173 APPARATUS

(**a**) *Infrared spectrophotometer.*—Recording, for operation in 2–15 μ region.

(b) *Water-repellent plate.*—Wash 3–4″ glass square thoroly with detergent, rinse, and dry with towel. Dip glass rod in organo-silicone compound and streak adhering liquid across plate. Repeat streaking several times. Rub plate with lens paper to distribute evenly; then rub with clean lens paper to remove excess. Plate is now ready for use. Wash plate with cold H_2O and dry with towel after each use. Plate can be reused as long as it remains nonwettable.

15.174 PREPARATION OF SAMPLE

Weigh 50 g sample of frozen dessert into 250 ml centrifuge bottle and heat to 60° in H_2O bath. Add 150 ml dioxane, shake vigorously 2 min., and centrifuge 10 min. at 1800 rpm. Decant and discard supernatant. Add 30 ml ether, and shake vigorously to break mass at bottom, using rod if necessary. Decant ether and repeat ether wash once. Heat in H_2O bath to remove residual ether. Add 30 ml 80° H_2O and shake vigorously 2 min. to dissolve or disperse residue.

15.175 SEPARATION OF GUM

Add 20 ml 50% trichloroacetic acid and heat to 60° in H_2O bath. Shake 1 min. and centrifuge 10 min. at 1200 rpm. Decant soln thru fast folded paper into second centrifuge bottle, and discard residue.

Fill centrifuge bottle with alcohol, add 1 ml satd NaCl soln, mix, and let stand until coagulation occurs. If ppt does not form, gums are absent. (Let opalescent solns stand overnight to facilitate pptn. Centrifuge, and discard if no ppt is present.) Centrifuge 10 min. at 1800 rpm and immediately decant and discard supernatant.

Purify pptd gum by adding 30 ml 80° H_2O and shake vigorously to dissolve or disperse ppt. Fill centrifuge bottle with alcohol, add 1 ml satd NaCl soln and 1 drop HCl (1+1), mix, let ppt coagulate, centrifuge 10 min. at 1800 rpm, decant, and discard supernatant. Repeat purification step twice.

15.176 DETECTION OF GUM

(a) *Chemical test for small amounts.*—Scrape small amount of pptd gum from centrifuge bottle with spatula and transfer to 50 ml beaker with ca 10 ml hot H_2O. Add 2 ml HCl and boil 5 min. Neutralize to multirange indicator paper, using 30% NaOH soln first and completing with 10% NaOH soln. Remove paper and continue as in **15.158**, beginning " . . . add 5 ml Benedict's soln . . ."

(b) *Chemical test for entire precipitate.*—If infrared spectrum is not desired, spectrum indicates presence of 2 or more gums, or pptd gum will not form film (as with karaya), proceed as in **15.158**, beginning "Add to residue in tube . . . "

(c) *Infrared method.*—(May not be applicable to mixts of gums.) Dissolve or disperse residue in 35 ml H_2O. Prep. film as in **15.177**, obtain infrared spectrum against air, and compare with spectra of reference gums.

15.177 PREPARATION OF GUM FILMS

Place H_2O repellent glass plate over 2″ opening on steam bath. Pour enough of aq. gum soln on plate to form circle ca 2″ diam. (Vol. required to produce film of sufficient area to cover light path of app. and of thickness to produce characteristic spectrum varies depending upon nature of gum, and its concn.) Heat plate until film is dry and remove film with forceps. If film sticks to plate, remove with razor blade or tissue lifter. Transfer film to beaker and dry 15–30 min. at 100°. (Excessive heating may char some gums.) Place piece of film between 2 salt plates and obtain infrared spectrum against air.

15.178 PREPARATION OF REFERENCE GUM FILMS

Disperse 0.2 g gum in 30 ml 80° H_2O and add 20 ml 50% trichloroacetic acid soln. Continue as in **15.175**, beginning "Shake 1 min. and centrifuge 10 min. at 1200 rpm."

15.179 Alginates in Chocolate Frozen Desserts—Official—*See* 12.044–12.045

15.180 Gelatin—Official

Using 10 g sample, proceed as in **15.040.**

15.181 Coloring Matters—Official

Curdle 150–200 g melted sample by adding equal vol. H_2O and 10–20 ml HOAc. Heat mixt. to 70–80°, with stirring, and let cool. Continue as in **15.045** and **15.123,** and in Chap. **35** for detection of oil-sol. coal-tar dyes and annatto.

15.182 Residual Phosphatase *(17)*—Official

Melt portion of sample and let it remain melted 1 hr or longer before testing. Then proceed as in **15.056** except as follows:

In *Step 3*, proceed as for milk in case of sherbets; for ice cream, substitute buffer substrate made by dissolving the $Na_2C_6H_5PO_4$ in mixt. of 4 parts of the Ba buffer, **15.054**(a)(1), and 1 part H_2O.

In *Step 6*, proceed as for milk in case of sherbets; for ice cream, ppt with 1.0 ml soln contg 4.5 g $ZnSO_4.7H_2O$ and 0.1 g $CuSO_4.5H_2O$/100 ml.

Controls are essential since phenols may be present from flavors or plastic containers.

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16. Eggs and Egg Products

16.001 **Collection and Preparation of Sample (*1*)—Procedure**

No simple rules can be made for collection of sample representative of av. of any particular lot of egg material, as conditions may differ widely. Experienced judgment must be used in each instance. For large lots, preferably draw several samples for sep. analyses rather than attempt to get one composite representative sample.

(a) *Liquid eggs.*—Obtain representative container or containers. Mix contents of container thoroly and draw ca 300 g. (Long-handle dipper or ladle serves well.) Keep sample in hermetically sealed jar in cool place. Report odor and appearance.

(b) *Frozen eggs.*—Obtain representative container or containers. Examine contents as to odor and appearance. (Condition of contents can be detd best by drilling to center of container with auger and noting odor as auger is withdrawn. If impossible to secure individual containers, samples may consist of composite of borings from contents of each container.) Take borings midway between center and circumference of top of can from at least 3 widely sepd parts and extend them as near to bottom of can as possible. Collect ca 300 g and keep sample in hermetically sealed jar in cool place and in frozen state if possible. Before analyzing, warm sample in bath held at $<50°$, and mix well.

(c) *Dried eggs.*—Obtain representative container or containers. For small packages, take entire parcel or parcels for sample. For boxes and barrels, remove top layer to depth of ca 6″ with scoop or other convenient instrument. Draw small quantities of sample totaling 300–500 g from accessible parts of container and place in hermetically sealed jar. Report odor and appearance. Prep. sample for analysis by mixing 3 times thru domestic flour sifter to thoroly break up lumps. Keep in hermetically sealed jar in cool place.

(d) *Flaked and drum-dried eggs.*—Collect sample as for powd. dried eggs. Report odor and appearance. Prep. albumen samples for analysis by grinding in mill to pass entirely thru No. 60 sieve, and prep. whole egg and yolk samples to pass entirely thru No. 20 sieve or as fine as is practicable. Keep in hermetically sealed jar in cool place.

Total Solids

Vacuum Method (2)—Official

16.002 APPARATUS

Vacuum oven.—Connected with pump to maintain partial vac. in oven with pressure equiv. to 25 mm or less of Hg and provided with thermometer passing into oven with bulb near samples. Connect H_2SO_4 gas-drying bottle to oven for admitting dry air to release vac.

16.003 DETERMINATION

(a) *Liquid eggs.*—Weigh accurately by difference, using weighing buret, ca 5 g sample, **16.001**(a) or (b), in covered dish previously dried at 98–100°, cooled in desiccator, and weighed soon after coming to room temp. Remove cover and evap. most of H_2O by heating on steam bath. Replace cover loosely and complete drying in vac. oven as in (b).

(b) *Dried eggs.*—Weigh ca 2 g sample, **16.001**(c) or (d), in covered dish previously dried at 98–100°, cooled in desiccator, and weighed soon after coming to room temp. Loosen cover (do not remove) and heat at 98–100° to constant wt (ca 5 hr) in vac. oven. Admit dry air into oven to bring to atmospheric pressure. Immediately tighten cover of dish, transfer to desiccator contg fresh efficient desiccant, and weigh soon after room temp. is reached. Report as % total solids.

Nitrogen (*3*)—Official

16.004 PREPARATION OF SAMPLE

(a) *Liquid eggs.*—Weigh 2–3 g well-mixed sample, **16.001**(a) or (b), by difference into 500 ml Kjeldahl flask.

(b) *Dried eggs.*—Transfer ca 1 g prepd. sample, **16.001**(c) or (d), accurately weighed, to 500 ml Kjeldahl flask.

16.005 DETERMINATION

Det. N as in **2.036.** Distill the NH_3 into 30–50 ml 0.1*N* std acid.

Water-Soluble Nitrogen and Crude Albumin Nitrogen in Liquid Eggs (*4*)—Official

16.006 PREPARATION OF SOLUTION

Weigh accurately, by difference, into 250 ml vol. flask contg 150 ml H_2O, ca 10 g well-mixed

sample **16.001**(**a**) or (**b**), and mix gently. Add 5 ml 0.01*N* HOAc for each g egg substance, dil. to mark with H_2O, shake gently, and filter thru 18.5 cm folded paper, covering filter with watch glass during filtration. If filtrate is cloudy, let filtration continue until drops of filtrate are clear, change receiver, return cloudy filtrate to filter, and proceed as in **16.007**.

16.007 DETERMINATION

(**a**) *Water-soluble nitrogen.*—Transfer 50 ml clear filtrate to 500 ml Kjeldahl flask, and det. N as in **2.036**. Calc. N and report as % H_2O-sol. N.

(**b**) *Crude albumin nitrogen.*—Transfer 100 ml clear filtrate to 200 ml vol. flask, add 15 ml NaCl soln (28 g NaCl dild to 300 ml), fill nearly to mark with alcohol, and mix. Cool to room temp., dil. to mark with alcohol, mix, and let stand overnight. Filter, transfer 100 ml filtrate to 500 ml Kjeldahl flask, and det. N as in **2.036**. Calc. % N, subtract it from % H_2O-sol. N, and report difference as % crude albumin N.

Fat By Acid Hydrolysis (*5*)—Official

16.008 PREPARATION OF SOLUTION

(**a**) *Liquid eggs.*—From well-mixed sample, **16.001**(**a**) or (**b**), weigh accurately by difference into Mojonnier fat-extn tube ca 2 g yolks, or 3 g whole eggs, or 5 g whites. Add slowly with vigorous shaking 10 ml HCl, set tube in H_2O bath heated to 70°, bring to boiling, and continue heating at boiling 30 min., shaking tube with care at 5 min. intervals. Remove tube from bath, add H_2O to nearly fill lower bulb of tube, and cool to room temp.

(**b**) *Dried eggs.*—Transfer 1 g well-mixed sample to fat-extn tube, add slowly 10 ml HCl (4+1), washing down any egg particles adhering to sides of tube, and proceed as in (**a**).

16.009 DETERMINATION

To extn tube contg treated sample, **16.008**, add 25 ml ether and mix. Add 25 ml redistd petr. ether (b.p. <60°), mix, and let stand until solvent layer is clear. Proceed as in **13.019**, beginning "Draw off as much as possible . . . " but omitting filtration.

Lipoids and Lipoid Phosphorus (P_2O_5) (*6*)—Official

16.010 REAGENTS

(**a**) *Mixed solvent.*—Equal vols $CHCl_3$ and absolute alcohol.

(**b**) *Alcoholic sodium hydroxide soln.*—Prep. CO_2-free soln by dissolving 100 g NaOH in 100 ml H_2O. Let stand until clear, or filter thru hardened paper previously soaked in alcohol. (5 ml of the NaOH soln contains ca 4 g NaOH). Dissolve 50 ml of this soln in 900 ml alcohol and dil. with alcohol to 1 L.

16.011 PREPARATION OF SOLUTION

(**a**) *Liquid eggs.*—Weigh accurately by difference ca 4 g well-mixed sample, **16.001**(**a**) or (**b**), into 100 ml vol. flask, and add 25 ml of the mixed solvent very slowly (dropwise) from pipet, shaking constantly until proteins coagulate and are then thoroly broken up. Add 60–65 ml more solvent and let stand 1 hr, shaking at 5 min. intervals. Dil. to mark with solvent, mix, and let mixt. stand until clear.

(**b**) *Dried eggs.*—Transfer 2 g well-mixed sample, **16.001**(**c**) or (**d**), to 100 ml vol. flask, add 85–90 ml of the mixed solvent, and let stand 1 hr, mixing at 5 min. intervals. Proceed as in (**a**).

16.012 DETERMINATION

(**a**) *Lipoids.*—Transfer 50 ml aliquot to 150 ml beaker and evap. ext. to dryness on steam bath. (Elec. fan or gentle blast of dry air may be used to hasten evapn.) Place beaker in oven 5–10 min. at 100° to remove any remaining H_2O. Dissolve dry ext. in 5–10 ml $CHCl_3$, and filter soln into weighed 100 ml Pyrex beaker thru pledget of cotton packed into stem of funnel, transferring all sol. ext. from bottom and sides of beaker with $CHCl_3$. Finally wash funnel and stem tip. (Filtrate should be clear.) Evap. $CHCl_3$ on steam bath and dry beaker and contents in oven at 100° to constant wt (ca 90 min). Let beaker stand in air to constant wt (ca 30 min.), weigh, and report % lipoids.

(**b**) *Lipoid phosphorus* (P_2O_5).—Dissolve dried lipoids in 2–3 ml $CHCl_3$, add 10–20 ml of the alc. NaOH soln, evap. to dryness on steam bath, using care to avoid spattering, and place beaker in oven 30 min. at 100° to remove any remaining H_2O. Transfer beaker while hot to muffle heated to 500° (faint red), and keep at this temp. 1 hr. Cool, add few drops H_2O, and break up charge with flat-end glass rod. Cover beaker with watch glass, add slowly 5 ml HNO_3 (1+3), mix, wash watch glass, and filter, collecting filtrate in 300 or 500 ml erlenmeyer. Thoroly wash charred material and filter paper with H_2O.

Det. P in filtrate as in **2.022**, using 20–50 ml of the molybdate soln. Report % lipoid P_2O_5 in eggs.

Cholesterol (*7*)—Official

Separation of Unsaponifiable Matter

16.013 REAGENTS

(**a**) *Concentrated potassium hydroxide soln.* Dissolve 60 g KOH in 40 ml H_2O.

(**b**) *Dilute potassium hydroxide soln.* Dissolve 10 g KOH in 1 L H_2O.

(c) *Ether.*—USP or ACS, peroxide-free. Test immediately before use.

(d) *Dried ether.*—Immediately before use shake peroxide-free ether with anhyd. $CaCl_2$ equal to 10% of the vol. of the ether, and filter.

(e) *Anhydrous sodium sulfate.*—Powder to pass No. 60 sieve.

16.014 APPARATUS

(a) *Separators.*—One 250 ml and one 500 ml. *Wash separators free of grease.* Should be ether-tight with stopcocks lubricated only with H_2O.

(b) *Filtration bell jar.*—Large enough to accommodate 300 ml erlenmeyer and provided with air-leak valve to control vac.

(c) *Fritted glass filter.*—Fine porosity fritted glass filter (Jena 11G3 or equiv.).

16.015 DETERMINATION

Weigh accurately into 125 ml erlenmeyer ca 2.5 g whole egg, 1.5 g yolk, 1 g dried whole egg, or 0.7 g dried yolk, and add 10 ml of the concd KOH soln. Cover with small watch glass and heat 3 hr on steam bath, swirling occasionally to disintegrate any large lumps. Cool until just warm, add 30 ml alcohol, and swirl until all insol. matter is *finely* dispersed. Add 50 ml ether, mix thoroly by swirling, and transfer to 500 ml separator. Wash flask with 2 more 50 ml portions ether and thoroly mix ether soln by swirling. Wash saponification flask with 100 ml of the dil. KOH soln, pour soln slowly into separator, while gently swirling liquid, and continue gentle swirling 10–15 sec. Let liquids sep. (ca 10 min.) and slowly drain soap soln into 250 ml separator, but do not draw off any small quantity of emulsion or insol. matter at interface. Rinse down sides of 500 ml separator with 10 ml of the dil. KOH soln and drain this into smaller separator. Add 50 ml ether to smaller separator and shake vigorously. After liquids sep., discard lower layer. Add ether layer to soln in large separator, rinsing 250 ml separator with 10 ml ether. Wash ether soln as before with 100 ml of the dil. KOH soln, keeping any insol. matter or emulsion in separator. Add to ether 20 ml HCl (1+4), swirl, add 100 ml H_2O, and swirl again. Discard acid washings.

Wash ether soln as before with 2 addnl 100 ml portions of the dil. KOH soln. Test portion of last washings for soap by acidifying with the HCl (acidified washings should be clear or only faintly turbid). If necessary, repeat washing with the dil. KOH soln until acidified washings are clear. Wash ether soln by successively swirling with 50 ml H_2O, 50 ml H_2O contg 0.5 ml 0.1N HCl, and 2 addnl 50 ml portions H_2O. Finally, drain as much H_2O as possible without loss of ether soln. Filter ether soln into dry 300 ml erlenmeyer thru 15 g layer Na_2SO_4 on fritted glass filter, using no suction for first few ml and then gentle suction for remainder. Rinse separator and filter successively with 10, 5, 5, and 5 ml ether. Rinse filter stem with ether, add porcelain chip to flask, and evap. ether on steam bath.

Dissolve residue in 20 ml dried ether, transfer ether soln thru small short-stem funnel to 50 ml erlenmeyer contg porcelain chip, and rinse with 10, 5, and 5 ml dried ether. Approx. unsaponifiable matter can be detd by collecting ether solns in flask previously dried and weighed as follows: Dry flask contg the chip, and similar flask used as counterpoise, 1 hr at 100–105°; remove from oven and place near balance 30 min.; weigh flask, using counterpoise. Evap. ether on steam bath. Wipe flask with clean towel, dry, and weigh with counterpoise as before. From wt unsaponifiable matter, deduct blank obtained from reagents used, detd by same procedure.

Determination of Cholesterol

16.016 REAGENTS

(a) *Ice.*—For 4 detns have available ca 3 gallons of crushed ice.

(b) *Bromine soln.*—Weigh, to 0.1 g, narrow-mouth, g-s. 25 ml flask contg 5 ml CCl_4. Add 0.6 ml Br from graduated 1.0 ml pipet, weigh again, and dil. with CCl_4 to calcd final concn of 0.22 $\pm$0.02 g Br/ml. Use reagent within 48 hr after prepn.

(c) *Acetic acid soln.*—Pipet 200 ml HOAc into 250 ml g-s. vol. flask; dil. to mark with H_2O, mix cautiously, dil. to mark, and mix again.

(d) *Asbestos.*—Prep. asbestos as in **29.038.**

(e) *Sand.*—Pass clean sand thru No. 60 sieve and treat with warm HCl until exts are practically colorless. Wash, dry, and ignite.

(f) *Sodium hypochlorite soln.*—Dissolve 88 g NaOH in 200 ml H_2O in wide-mouth 3 L flask. Add ca 1.5 L crushed ice and pass in Cl until 71 g is absorbed; dil. to 2 L and store in dark bottles in refrigerator. (Soln should be alk. to phthln.) Before use, check concn of available Cl as follows: Pipet 5 ml into flask contg soln of 2 g KI in 100 ml H_2O, add 5 ml 6N HCl, and titr. with 0.1N $Na_2S_2O_3$. Soln should be equiv. in available Cl to 0.95–1.05N NaOCl. Reagent or commercial NaOCl soln, 5%, checked for concn as above, is also satisfactory.

(g) *Sodium formate soln.*—Prep. aq. soln of $NaCHO_2$ contg 0.5 g of the salt/ml.

(h) *Hydrochloric acid.*—Approx. 6N; mix 520 ml HCl (not <35% HCl by wt) with H_2O and dil. to 1 L.

(i) *Methyl red indicator.*—Dissolve 0.5 g Me red in 50 ml alcohol, dil. to 100 ml with H_2O, and filter. Since soln must be free from insol. matter, refilter immediately before use if necessary.

(j) *Potassium iodide soln.*—20%. (Soln must be colorless when acidified with HCl.)

(k) *Starch soln.*—1% soln of sol. starch.

(l) *Sodium thiosulfate soln.*—0.02*N*. Prep. from $Na_2S_2O_3.5H_2O$ and CO_2-free H_2O as in **42.035**, and add 1% amyl alcohol. (Soln usually retains titer for months.) Stdze with exact 0.02*N* soln of pure KIO_3 as follows: To g-s. 125 ml erlenmeyer add 5 ml of the KI soln, 10 ml H_2O, 1.5 g $NaHCO_3$, and 5 ml 6*N* HCl. Mix, add 25–30 ml of the KIO_3 soln, and titr. at once with the $Na_2S_2O_3$ soln, using starch soln as indicator.

(m) *Potassium hydroxide soln.*—Dissolve 10 g KOH in 10 ml H_2O.

(n) *Ammonium molybdate soln.*—Dissolve 5 g $(NH_4)_6Mo_7O_{24}.4H_2O$ in 100 ml H_2O.

16.017 APPARATUS

(a) *Ice bath.*—Container holding ca 4 L, 10–15 cm deep, filled with crushed ice.

(b) *Mohr pipets.*—One graduated to 0.01 ml; one graduated to 0.1 ml.

(c) *Filtration bell jar.*—Size to accommodate 300 ml erlenmeyer, connected to vac. by 2-way stopcock.

(d) *Device for filtering at 0°.*—Filter tube of Knorr extn tube style, ca 20 mm i.d., with body ca 11 cm long and stem 6–8 mm o. d., ca 10 cm long, provided with removable, close fitting perforated Ni, monel metal, glass, or porcelain disk at bottom of larger tube. (Allihn fritted glass filter, coarse porosity, Ace Glass Co. No. 8571 with 10 cm stem is satisfactory.)

Remove stem at apex from 60° Bunsen funnel, 11 cm diam. Enlarge opening at apex to ca 1 cm diam. by grinding or grating off glass. Cut ca 1 cm from end of 1 hole rubber stopper of size that fits snugly in opening of funnel. Pass stem of filter, tube thru stopper in funnel apex and then thru stopper in bell jar.

Prep. in filter tube mat of the asbestos 6–8 mm thick and cover with ca 12 mm layer of the sand.

16.018 DETERMINATION

Pack the Br soln and 25 ml graduated cylinder in ice. Pack ice around filter tubes, taking care none gets into filters. Cool the HOAc soln to ca −5° in ice-NaCl mixt.

Wash down sides of 50 ml erlenmeyers contg unsaponifiable matter, while rotating them, with 2.0 ml absolute ether delivered from Mohr pipet; stopper with cork, swirl until porcelain chips no longer stick to flasks, and pack flasks in ice bath up to necks at least 10 min. To one of flasks add, from Mohr pipet, 0.20 ml of the cold Br soln, mix by swirling, stopper, and replace in ice bath. Start this procedure at 3 min. intervals with other flasks (4 detns can be made at one time if bell jars are available).

After 10 min., add rapidly 15 ml of the HOAc soln from the cold 25 ml cylinder, swirl 3 min. while holding in ice-H_2O, and replace in ice bath 10 min. With suction on, pour all mixt. down stirring rod into filter tube, leaving rod in filter. Wash down sides of flask with 5 ml of the cold HOAc soln and replace in ice bath. When liquid in filter just recedes below surface of sand, add the HOAc from flask. Repeat washing in like manner with 5 ml of the HOAc soln and suck filter free of excess liquid. Wash flask and filter with cold H_2O, filling filter tube ca 3 times. Drain flask thoroly and apply suction to filter until drops of H_2O cease to fall from stem. Remove ice pack from around filter tube and discard filtrate and washings.

Place 300 ml erlenmeyer under filter so that stem projects well into neck of flask. Wash filter tube and filter with 10 ml alcohol; 10, 5, and 5 ml portions ether; and finally with 10 ml alcohol, gently stirring sand with each portion of solvent and letting mixt. stand ca 1 min. before applying suction. Wash stem of filter with few ml ether, add 1 ml of the KOH soln, mix, and wash down sides of flask with 5 ml ether. Evap. ether and alcohol completely on steam bath, finally using stream of clean air to remove last of alcohol vapors.

Add 40 ml hot H_2O to residual alk. liquid, mix, and neutralize alkali with 6*N* HCl, using *1 drop* Me red. (This neutralization need be only approx.) Add 10 g NaCl, 3 g $NaH_2PO_4.H_2O$, and 20 ml of the NaOCl soln. Bring soln just to vigorous boiling, remove from heat, and add immediately, with care, 5 ml of the $NaCHO_2$ soln. Cool, and dil. to ca 150 ml with H_2O. Add 5 ml of the KI soln, 1 or 2 drops of the NH_4 molybdate soln, **16.016(n)**, and 25 ml of the 6*N* HCl. Titr. rapidly at once with the $Na_2S_2O_3$ soln, using starch soln as indicator. Correct titer for blank detn on reagents, starting at point where KOH soln is added to alcohol-ether soln.

$$0.55 + 0.688 \times \text{ml } 0.02N\ Na_2S_2O_3 = \text{cholesterol (mg)}.$$

Total Phosphorus (P_2O_5) (*8*)—Official

16.019 PREPARATION OF SOLUTION

(a) *Liquid eggs.*—From well-mixed sample, **16.001(a)** or **(b)**, weigh accurately, by difference, into 250 ml Pyrex beaker, ca 2 g yolks, 4 g whole eggs, or 10 g whites. Add 20 ml 10% Na_2CO_3 soln and evap. to dryness on hot plate or in oven overnight at 100–105°. Transfer beaker while hot to muffle at 500° (faint red), and keep at this temp. 1 hr. Cool, add few drops H_2O, break up charge with flat-end glass rod, cover beaker with watch glass, add slowly while stirring 10 ml HNO_3 (1+3), mix, and wash watch glass and filter, collecting filtrate in 300 or 500 ml erlenmeyer.

Thoroly wash charred material and filter with H_2O.

(**b**) *Dried eggs.*—Transfer 1 g well-mixed sample, **16.001**(**c**) or (**d**), to 150 ml Pyrex beaker, add 20 ml 10% Na_2CO_3 soln, and proceed as in (**a**).

16.020 DETERMINATION

Det. P_2O_5 in prepd filtrate as in **2.022,** using 40–50 ml of the molybdate soln. Report as total P_2O_5.

Chlorine (*9*)—Official

16.021 *Method I.*

(**a**) *Liquid eggs (in absence of added salt).*—From well-mixed sample, **16.001**(**a**) or (**b**), weigh accurately, by difference, into 150 ml Pyrex beaker, ca 4 g yolks, 7 g whole eggs, or 10 g whites; add 20 ml 10% Na_2CO_3 soln, mix, and evap. to dryness on hot plate or overnight in oven at 100°. Transfer beaker while hot to muffle at 500° (faint red), and keep at that temp. 1 hr. Cool, add few drops H_2O, and break up charge with glass rod. Add 50 ml H_2O, cover beaker with watch glass, add slowly 20 ml HNO_3 (1+3), and wash watch glass. Mix, filter, and wash charred material and filter paper thoroly with H_2O. Proceed by one of following alternatives:

(1) To combined filtrate and washings add known vol. 0.1*N* $AgNO_3$ in slight excess and proceed as in **6.068.**

(2) Collect filtrate and washings in 250 ml vol. flask, keeping total vol. filtrate to 180 ml or less. Add known vol. 0.1*N* $AgNO_3$ in slight excess and dil. to vol. Filter, and using aliquot of filtrate, proceed as in **6.068,** beginning "add 5 ml of the ferric indicator . . ."

(**b**) *Liquid eggs (in presence of added salt).*—From well-mixed sample, **16.001**(**a**) or (**b**), weigh 1–2 g accurately, by difference, into 150 ml Pyrex beaker, and proceed as in (**a**).

(**c**) *Dried eggs.*—From well-mixed sample, **16.001**(**c**) or (**d**), transfer, to 150 ml Pyrex beaker, 2 g whole eggs or yolks, or 1 g whites, and proceed as in (**a**).

16.022 *Method II.* (*10*)

From well-mixed sample, **16.001**(**a**), (**b**), (**c**), or (**d**), weigh accurately, by difference, ca 4 g yolks, 7 g whole eggs, or 10 g whites; or transfer 2 g dried whole eggs or yolks, or 1 g dried whites, to 300 ml erlenmeyer. Add known vol. 0.1*N* $AgNO_3$ in slight excess and 20 ml HNO_3, and place mixt. on steam bath 15–30 min. Add 15 ml 5% $KMnO_4$ soln and let stand 60–90 min. longer on steam bath. Cool to 25° or less; add 75 ml H_2O and 1 ml *nitrobenzene* (or 1 ml for each 50 mg NaCl present); stopper flask, and shake vigorously to coagulate ppt. Add 5 ml satd ferric alum indicator and titr. with 0.1*N* thiocyanate soln to end point that persists after soln stands 15 min. (Make titrn at 25° or below; soln is yellow-green before end point and yellow-orange at end point.) At first permanent color change, note buret reading and time; stopper flask, shake vigorously, and let stand 15 min. If soln fades, add thiocyanate soln in half-drop portions until end point color reappears (*11*). From ml $AgNO_3$ used, calc. NaCl after deducting blank detd on reagents, using ca 0.25 g sucrose instead of egg.

Carotene (*12*)—First Action

16.023 PREPARATION OF STANDARD CAROTENE SOLUTION

Prep. solns contg 0.5, 1.0, 2.0, 3.0, 4.0, and 5.0 mmg β-carotene/ml by weighing 0.25, 0.50, 1.0, 1.5, 2.0, and 2.5 g std 0.05% β-carotene in oil soln into series of 250 ml vol. flasks, and dilg to vol. with acetone. Solns are stable at least 1 week in dark under refrigeration. Equiv. amounts of std β-carotene soln of different strength may be used.

16.024 PREPARATION OF STANDARD CURVE

Det. absorbance of dild std solns as soon as possible with spectrophotometer at 450 mμ or with instrument with suitable filter system such as Klett photometer with No. 44 filter, Evelyn photoelec. colorimeter with 440 filter, or with Cenco-Sanford-Sheard Photelometer, Industrial Type B-2 with 410 filter. Plot mmg β-carotene against % transmittance, omitting values >90% or <10%, on semilog paper or against absorbance on plain coordinate paper.

16.025 DETERMINATION

Weigh sample contg ca 1.0 g egg yolk solids (1 g dried yolk, 2.5 g liquid yolk, 5.0 g liquid whole egg, or equiv.) into 150 ml beaker. Add ca 1–2 ml acetone and stir to smooth paste. Add ca 50 ml acetone, mix, and filter. Add ca 2.5 ml H_2O before the acetone to products contg sugar or salt. Wash material onto Whatman No. 4 filter paper or equiv. with successive small portions of acetone, catching filtrate in g-s. 100 ml vol. flask. Dil. to vol. with acetone. Det. % transmittance or absorbance as soon as possible. Calc. mmg β-carotene/g sample.

Dextrose and Sucrose (*13*)—Official

16.026 PREPARATION OF SOLUTION

(**a**) *Liquid eggs.*—Weigh accurately, by difference, ca 25 g well-mixed sample, **16.001**(**a**) or (**b**), into 250 ml vol. flask contg 1 g $CaCO_3$ and 50 ml 5% NaCl soln. Add 130 ml alcohol with continuous mixing. Let stand few min. for gas bubbles to rise to surface, cool to room temp., dil. to mark with H_2O, mix, and filter (18.5 cm folded paper).

Transfer 150 ml filtrate to 250 ml beaker and evap. to 20–30 ml to remove alcohol. Cool, and wash with H_2O into 100 ml vol. flask, holding vol. to 80–90 ml. Add dry powd. *phosphotungstic acid* in small quantities in slight excess to ppt any protein, mix, let stand few min. for gas bubbles to rise to surface, dil. to mark with H_2O, mix, and filter. To filtrate add, in very small portions, enough dry powd. KCl to ppt any excess phosphotungstic acid, filter if necessary, and test filtrate for complete pptn.

To correct for error due to vol. occupied by ppt in samples contg added sucrose, repeat detn, weighing same quantity of sample into 500 ml vol. flask contg 1 g $CaCO_3$ and 100 ml 5% NaCl soln. Add 260 ml alcohol with continuous mixing. Let stand few min. for gas bubbles to rise to surface, cool to room temp., dil. to mark with H_2O, mix, and filter thru 18.5 cm folded paper. Transfer 300 ml filtrate to 400 ml beaker, evap. to 20–30 ml, and proceed as above. To obtain quantity of sucrose, subtract % sucrose obtained in 250 ml diln detn from twice the % obtained in 500 ml diln detn.

(**b**) *Dried eggs.*—From well-mixed sample, **16.001**(**c**) or (**d**), transfer 2.5 g whites, or 10 g yolks or whole eggs, to 250 ml vol. flask contg 1 g $CaCO_3$ and 50 ml 5% NaCl soln, and let stand 1 hr, mixing at 5 min. intervals. Add 130 ml alcohol with continuous mixing, and proceed as in (**a**), beginning with third sentence.

16.027 DETERMINATION

(**a**) *Reducing sugars direct.*—Transfer 25 ml prepd filtrate to 400 ml beaker, and proceed as in **29.039.** Report as % dextrose.

(**b**) *Reducing sugars invert.*—Transfer 50 ml prepd filtrate to 100 ml vol. flask, and invert sucrose as in **29.026**(**b**) or (**c**). Neutralize with NaOH soln, cool to room temp., and dil. to mark with H_2O. Transfer 50 ml (or less) to 400 ml beaker, and proceed as in **29.039.** Deduct % invert sugar obtained before inversion from that obtained after inversion, multiply difference by 0.95, and report as % sucrose.

Glycerol (*14*)

Qualitative Test—Procedure

16.028 REAGENT

Fuchsin-bisulfite soln.—Dissolve 0.2 g basic fuchsin in 120 ml hot H_2O and cool; add soln of 2 g anhyd. Na_2SO_3 in 20 ml H_2O, and then 2 ml HCl. Dil. soln with H_2O to 200 ml and let stand 1 hr.

16.029 DETECTION

Add 20 ml alcohol to ca 5 g sample in erlenmeyer or beaker-flask, shake vigorously, and filter thru 12.5 cm fluted paper. Evap. filtrate rapidly until no odor of alcohol is perceptible, cool, and add 3–4 drops H_2O and then 10–15 ml anhyd. ether. Mix solns carefully, let sep., and pour off as much of ether layer as possible, disregarding cloudiness in this layer. Shake well with two 10 ml portions anhyd. ether, pouring off ether carefully in each case. (Vol. aq. soln should be not <0.4–0.5 ml.) Evap. remaining liquid on steam bath to 0.1–0.2 ml. Cool, and add 15 ml mixt. of equal vols absolute alcohol and $CHCl_3$. Cool, shake, and let stand 5 min.

Shake, and filter thru fluted paper into $6 \times 1''$ Pyrex test tube. Evap. filtrate rapidly (small flame and current of air is convenient) until no odor of $CHCl_3$ or alcohol is perceptible. Add several g powd. $KHSO_4$ and insert stopper with glass tube leading into 2 ml H_2O in test tube immersed in ice-H_2O. Heat with small flame until frothing ceases and contents of tube are liquid. Remove receiver, add immediately 4–5 drops of the fuchsin-bisulfite reagent, and warm to room temp. In presence of glycerol, strong pink color (due to acrolein) develops within 1 min. and becomes deep violet within 5 min.

Quantitative Method (15)—Official

16.030 REAGENTS

(**a**) *Sodium tungstate soln.*—Dissolve 10 g $Na_2WO_4.2H_2O$ in H_2O and dil. to 100 ml.

(**b**) *Potassium periodate soln.*—0.02*M*. Dissolve 4.6 g KIO_4 in ca 500 ml hot H_2O, dil. to ca 900 ml with H_2O, cool to room temp., and dil. to 1 L. Test for alky by adding 0.02*N* H_2SO_4 to 25 ml of the soln contg bromocresol purple, (**c**). Do not use if >1 drop of the acid is required to give yellow acid color.

(**c**) *Bromocresol purple indicator.*—Dissolve 0.1 g bromocresol purple in 100 ml alcohol and filter if necessary.

(**d**) *Calcium oxide, powd.*—Reagent grade.

16.031 DETERMINATION

(**a**) *Eggs with no added sugars.*—Weigh accurately, by difference, ca 2 g well-mixed sample, **16.001**(**a**) or (**b**), into 100 ml vol. flask contg 50–75 ml H_2O. Mix and add 2.0 ml of the Na_2WO_4 soln. Add slowly 2.0 ml 1*N* H_2SO_4 with continuous mixing. Dil. to mark with H_2O, mix well, and filter (18.5 cm folded paper). Transfer aliquot of filtrate contg not >40 mg glycerol to 300 ml erlenmeyer, and dil. with H_2O to 20 ml if necessary. Add 2 ml 10% NaOH soln, heat to boiling, and boil 30 sec. Cool slightly, add 3 drops of the bromocresol purple, neutralize with 1*N* H_2SO_4 (use buret), and add 1–2 drops excess. Boil 1 min., cool to room temp., and carefully neutralize with 0.02*N* NaOH, titrg to light purple shade.

Transfer neutral soln quantitatively to 100 ml

vol. flask, restricting total vol. to <50 ml. (As aid, mark side of flask to indicate vol. of ca 45 ml.) If necessary, add more $0.02N$ NaOH to maintain light but definite purple. Continue as in **34.060**(b), beginning "add 50 ml of the KIO_4 soln." and using **34.061**(a) for the detn.

Excess periodate must be present after oxidation. If periodate test is negative, repeat detn, using small aliquots.

(b) *Eggs containing added sugars.*—Prep. sample soln as in (a), using ca 2 g sample.

Transfer aliquot of filtrate contg not >40 mg glycerol to 400 ml beaker. Adjust vol. to 20 ml by evapn on steam bath or by addn of H_2O. Add 0.5 g powd. CaO, mix, and let stand 30 min. at room temp. with occasional mixing. Add 25 ml alcohol, mix, and filter with suction, using büchner and 7 cm S&S 597 paper or equiv. Rinse beaker, funnel, and paper with several portions of alcohol. Transfer as much as possible of the residue to the paper but do not attempt to remove film of lime salts adhering to beaker. Transfer filtrate quantitatively to original 400 ml beaker, rinsing flask with several portions of H_2O. Evap. filtrate on steam bath to ca 10 ml. Filter thru 9 cm S&S 597 paper or equiv., collecting filtrate in 300 ml erlenmeyer. Rinse beaker, funnel, and paper with small quantities of H_2O, restricting total filtrate vol. to 25 ml or less. Add 1 ml 10% NaOH soln to filtrate and complete detn as in (a), beginning "heat to boiling, and boil 30 sec."

Acidity of Ether Extract (*16*)—Official

(Not applicable to egg white)

16.032 REAGENTS

(a) *Benzene.*—Use best quality available. If not neutral, titr. 50 ml with the $0.05N$ Na ethylate, (b), and correct subsequent results accordingly.

(b) *Sodium ethylate.*—$0.05N$. Dissolve piece of metallic Na, ca 1 ml in vol., in 800 ml absolute alcohol. Titr. 10 ml $0.1N$ HCl with this soln and add calcd vol. absolute alcohol to make soln $0.05N$. Stdze against $0.1N$ HCl on day soln is used.

16.033 DETERMINATION

(a) *Dried eggs.*—Weigh 2 g dried eggs into small lipped erlenmeyer, add 30 ml ether, and mix well. After ether layer clears, decant thru small filter paper into tared flask. Repeat extn with three 20 ml portions ether. Evap. ether on steam bath and dry ext. 15 min. at 100°. Cool, weigh, dissolve in 30 ml of the benzene, add 3–4 drops phthln, and titr. with the Na ethylate soln. (End point is reached when yellow changes to orange.) Report as ml $0.05N$ Na ethylate required/g ether ext.

(b) *Liquid eggs.*—Weigh ca 8 g liquid eggs into 9 cm lipped porcelain evapg dish, and dry at 55° under pressure not >125 mm Hg until eggs are thoroly dry (ca 5 hr). Grind dried eggs in evapg dish with small pestle, and proceed as in (a), beginning "add 30 ml ether, and mix well."

Lactic Acid (*17*)—Official

(If succinic acid is also to be detd, proceed as in **16.037–16.040.**)

16.034 PREPARATION OF SOLUTION

(a) *Liquid or frozen eggs.*—Transfer 40 g sample to tared 300 ml erlenmeyer, add ca 75 ml H_2O, and shake thoroly. Add 15 ml $1N$ H_2SO_4 and 25 ml *20% phosphotungstic acid soln*, dil. to 200 g with H_2O, shake ca 1 min., and filter thru folded paper.

(b) *Dried eggs.*—Mix 10 g sample and 100 ml H_2O into uniform paste with stirring rod and add, with constant stirring, 10 ml $1N$ H_2SO_4, followed by 15 ml *20% phosphotungstic acid soln.* Transfer mixt. with H_2O to tared 300 ml erlenmeyer, dil. to 200 g with H_2O, shake ca 1 min., and filter thru folded paper.

Weigh 100 g filtrate obtained as in (a) or (b) into 250 ml beaker and evap. to ca 25 ml. Transfer material to liquid extractor with 25 ml H_2O and proceed as in **15.012.** Report lactic acid in terms of mg/100 g, making no correction for insol. solids in portion taken for analysis.

Volatile Fatty Acids (*17*)—Official

16.035 PREPARATION OF SOLUTION

(a) *Liquid or frozen eggs.*—Weigh 80 g into tared 500 ml erlenmeyer, add ca 150 ml H_2O, and shake vigorously.

(b) *Dried eggs.*—Weigh 25 g into 250 ml beaker, and with heavy stirring rod make into smooth paste with H_2O. Transfer mixt. to tared 500 ml erlenmeyer, using ca 200 ml H_2O.

Add 25 ml $1N$ H_2SO_4 to mixt. obtained as in (a) or (b) and shake ca 1 min. Add *20% phosphotungstic acid soln* (40 ml usually enough to give clear filtrate), dil. to 350 g with H_2O, and shake 1 min. Filter thru 24 cm folded paper.

16.036 DETERMINATION

Pipet 150 ml filtrate, **16.035,** (equiv. to 150 g; sp. gr. is ca 1.00) into distn flask of app. (Fig. 31, page 236) and proceed as in **18.019–18.021.**

To calc. quantities of acetic, propionic, and butyric acids in aliquot in distn flask divide titrns (calcd in mg of the acid) by 0.57, 0.81, and 0.92, resp. (fractions distd in first 200 ml portion). For dried eggs multiply by 9.34 and for liquid eggs by 2.92 to obtain mg acid/100 g sample.

To calc. quantity of formic acid in aliquot in

distn flask divide mg detd in distillate by 0.24 (fraction distd in second 200 ml portion) and multiply by 2.92 to obtain mg acid in 100 g liquid eggs and by 9.34 to obtain mg acid in 100 g dried eggs.

Succinic Acid (*18*)—Official

16.037 APPARATUS

(**a**) *Continuous extractor.*—*See* Fig. 26, p. 187.

(**b**) *Chromatographic tube.*—Approx. 17 mm o. d.×250 mm, plugged at constricted end with either cotton or glass wool.

16.038 REAGENTS

(**a**) *Solvent.*—*Tert*-butanol-$CHCl_3$ (1+4).

(**b**) *Glycerol indicator soln.*—Dissolve 75 mg mono NH_4 salt of 3-(4-anilino-1-naphthylazo)-2,7-naphthalenedisulfonic acid (Alphamine Red R, Eastman No. 6410) in 50 ml glycerol, warming on steam bath.

(**c**) *Phenol red indicator.*—Rub 100 mg phenolsulfonphthalein in mortar with 5.7 ml 0.05*N* NaOH until dissolved; then dil. to 100 ml with H_2O.

16.039 PREPARATION OF SOLUTION

(**a**) *Liquid or frozen eggs.*—Weigh 200 g sample into 1 L erlenmeyer, add 500 ml H_2O, and mix well, avoiding violent shaking; add 75 ml 1*N* H_2SO_4 and mix well. Add 125 ml *20% phosphotungstic acid soln*, dil. to 1 kg with H_2O, and shake 1 min. Divide between two 24 cm rapid folded filter papers. Transfer 250 ml filtrate to 400 ml beaker, evap. to ca 50 ml, add another 250 ml to same beaker, and evap. to 10 ml. If material starts to bump when vol. becomes low, use steam bath.

If <200 g sample is available, take 100 g, filter the phosphotungstic acid ppt on büchner with suction, and use total weighed filtrate for detn.

(**b**) *Dried eggs.*—Weigh 50 g sample into 400 ml beaker, and with heavy stirring rod make into smooth paste with H_2O. Transfer to 1 L erlenmeyer and add enough H_2O to make total wt of 700 g. Add 50 ml 1*N* H_2SO_4 and mix well. Add 75 ml *20% phosphotungstic acid soln* and proceed as in (**a**), beginning "dil. to 1 kg with H_2O . . . "

16.040 EXTRACTION

Place 15 g $(NH_4)_2SO_4$ in dry extractor. Transfer evapd material, **16.039**(**a**) or (**b**), to inner tube of extractor by washing thru small funnel with enough H_2O to make total vol. of 40 ml, add 0.5 ml H_2SO_4 (1+1), and mix by raising and lowering inner tube. Rinse beaker with 50 ml ether and pour rinsings into inner tube of extractor. Connect efficient condenser to extractor and proceed with extn as in **15.012**, placing 150 ml ether in extn flask and extg 3 hr or as long as necessary for complete extn.

(To det. time necessary for complete extn, transfer ca 20 mg *succinic acid,* accurately weighed, to extractor contg 20 g $(NH_4)_2SO_4$, add enough H_2O to give total vol. of 40 ml, and proceed with extn as above. After 3 hr add 10 ml H_2O to extn flask, evap. ether on steam bath, and titr. If recovery is <95%, ext. another 20 mg succinic acid for longer period and titr. Continue until 95% recovery is obtained, and use this period of extn for detn.)

To flask contg ether ext. add 5 ml H_2O and evap. ether on steam bath. Using graduated 5 ml pipet, neutralize contents of flask with satd $Ba(OH)_2$ soln, using phthln. Adjust vol. to 20 ml with H_2O, add 90 ml alcohol, heat almost to boiling on steam bath, and cool. Add ca 0.5 g filter-aid and filter with suction thru suitable filter, such as Caldwell crucible charged with thin layer of asbestos overlaid with small quantity of filter-aid added from suspension in H_2O. Rinse flask with 3 portions of alcohol (9+2), transferring each rinsing to crucible and sucking dry before adding another portion. Reserve filtrate for detn of lactic acid, **15.013**, beginning line 8, "To expel alcohol, evap . . . " using entire filtrate. Transfer contents of crucible to 100 ml beaker with 15–20 ml H_2O, acidify to *Congo red paper* with 1–2 drops H_2SO_4 (1+1), warm on steam bath, and refilter with suction, rinsing beaker with three 10 ml portions H_2O, transferring each rinsing to crucible, and sucking dry before adding another. Evap. filtrate to ca 5 ml, neutralize with 1*N* NaOH, transfer with H_2O to 50 ml beaker, and evap. to dryness on steam bath.

16.041 PREPARATION OF PARTITION COLUMN

Place 5 g H_2SiO_3 in mortar and add 0.5 ml of the freshly prepd glycerol indicator soln. (More soln may be necessary if it has stood several weeks.) Then add max. quantity of glycerol (1+1) that gel will hold without becoming sticky (usually 1–3 ml) and 1 drop (ca 0.05 ml) ca 1*N* NH_4OH. Grind into uniform powder with pestle, make into slurry with ca 30 ml of the solvent, and transfer to the chromatographic tube, which is clamped vertically. Apply 5–10 lbs air pressure to top of tube until solvent just disappears into top of gel; release pressure, add 1 ml $CHCl_3$ contg ca 5 mg HOAc, and again apply pressure until solvent just disappears into gel. Release pressure, add 5 ml of the solvent, and once more apply pressure just long enough for solvent to disappear into gel. (Pressure should never be left on with no liquid above gel; gel would then dry and crack, becoming useless.)

16.042 DETERMINATION

To dry residue of Na succinate, **16.040,** add 2 ml of the solvent and 3 drops H_2SO_4 (1+1), and stir with glass rod until all particles are moistened (material should be acid to Congo red paper). Add anhyd. Na_2SO_4 in 0.5 g portions until material is dry (not gummy), stir, and decant onto prepd partition column, pouring it slowly down side of tube in order to keep surface of gel level. Apply pressure until solvent just disappears into gel. Again wash beaker with 1 ml of the solvent, pour onto column, and with stirring rod transfer residue in beaker to column. Wash beaker with another 1 ml solvent, transfer to column, wash inside of tube with 1 ml solvent, and apply pressure until solvent just disappears into gel. Fill tube with solvent and apply pressure. Let HOAc band pass out of tube. When front of succinic acid band reaches constricted portion of tube, start collecting the eluate in 50 ml graduated cylinder. Continue collecting until band has passed entirely from tube or until lower edge of any following band reaches 2–5 mm above narrowest portion of constriction of tube, and until enough eluate collects to insure removal of succinic acid from tube. (Light placed adjacent to tube, but not so close as to heat it, increases visibility of bands.)

(To insure complete removal of succinic acid from column when there is no following band, det. total quantity of eluate to be collected by prepg soln of known quantity of Na succinate, transferring free acid to column, eluting, etc., as above, and titrg 25 ml and successive 10 ml fractions of eluate until last fraction requires <0.2 ml $0.01N$ alkali to neutralize. Total quantity of eluate required is quantity to collect in detn.)

Add 10 ml H_2O to flask and titr. with $0.01N$ $Ba(OH)_2$, using the phenol red indicator, in CO_2-free atmosphere. As end point approaches, stopper flask and shake vigorously to ext. acid completely from solvent phase. 1 ml $0.01N$ $Ba(OH)_2$ =0.59 mg succinic acid. If crystallographic identification of Ba succinate (*19*) is not desired, $0.01N$ NaOH may be used for titrn.

Water-Insoluble Fatty Acids (*20*)—First Action

16.043 PREPARATION OF SOLUTION

(**a**) *Liquid or frozen eggs.*—Weigh 10 g prepd sample, **16.001**(**a**) or (**b**), into 250 ml centrifuge bottle, add 25 ml H_2O, and mix. Add 20 ml alcohol, shake vigorously, and add 50 ml ether.

(**b**) *Dried eggs.*—Weigh 2 g prepd sample, **16.001**(**c**), into 100 ml beaker and stir to uniform paste with small quantity of H_2O, using heavy stirring rod. Transfer material to 250 ml centrifuge bottle with H_2O, using total of 25 ml for entire operation, and shake vigorously. Rinse beaker with 25 ml alcohol, transfer rinsings to centrifuge bottle, shake vigorously, and add 50 ml ether.

16.044 DETERMINATION

Proceed as in **15.125.**

Pyoverdine (*21*)—First Action

(Protect from daylight and other sources of ultraviolet light. Incandescent light and pink fluorescent light may be used. Avoid dissolved metals and contact with rubber.)

16.045 REAGENTS

(**a**) *Alcohol.*—95% USP, redistd from glass.

(**b**) *Chloride buffer soln.*—pH 1. Dry ca 50 g KCl at 120° overnight. Weigh 37.28 g into 500 ml vol. flask, dissolve in H_2O, and dil. to mark. Add 50 ml of this $1N$ KCl to 97 ml stdzd $1N$ HCl in 200 ml vol. flask, and dil. to mark with H_2O.

(**c**) *Potassium acid phthalate soln.*—$0.1M$. Dry ca 20 g at 120° overnight. Weigh 10.21 g into 500 ml vol. flask, dissolve in H_2O, and dil. to mark with H_2O.

(**d**) *Riboflavin std soln.*—0.50 mmg/ml, pH 4. Pipet 5 ml riboflavin stock soln II, **39.034**(**b**), into 100 ml vol. flask, add 50 ml $0.1M$ K acid phthalate, and dil. to vol. with H_2O. Prep. weekly and store in refrigerator.

16.046 DETERMINATION

Weigh 50 ± 0.1 g sample, thawed on day of analysis, into 250 ml beaker. Add ca 90 ml alcohol and disperse with prompt and thoro stirring. Transfer to 200 ml Kohlrausch vol. flask with alcohol, mix thoroly, and dil. to vol. with alcohol. Pour into dry 250 ml centrifuge bottle and centrifuge ca 15 min. at ca 1000 rpm. Pipet 100 ml supernatant into 250 ml separator. Add ca 125 ml $CHCl_3$ and shake by hand ca 100 strokes. Let emulsion stand in dark at least 6 hr, but not >24 hr. Drain and discard lower $CHCl_3$ layer contg fatty material and alcohol. Add ca 100 ml $CHCl_3$ and repeat extn, draining any residual emulsion into 50 ml vol. flask. Pass stream of air into flask until most or all of $CHCl_3$ evaps, and pipet in 10 ml of the pH 1 buffer. Transfer remainder of aq. layer to flask, using H_2O, and dil. to vol. If soln is cloudy, centrifuge briefly and pour off supernatant (1 ml is equiv. to 0.5 g sample).

Measure fluorescence of test and std solns in photofluorometer, using Corning 5874 as primary filter, and Corning 3486 as secondary filter. Calc. concn of pyoverdine expressed as mmg riboflavin /100 g egg $= 2 \times C \times R/S$, where 2 is derived from concn sample in final ext., C = concn riboflavin std in mmg/100 ml, R = reading on sample, and S = reading on std riboflavin soln.

16.047 **Quaternary Ammonium Compounds**—*See* **27.060**(d)

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(*3*) Ibid. **8**, 601(1925).
(*4*) Ibid. **15**, 344(1932).
(*5*) Ibid. **8**, 601(1925); **16**, 298(1933).
(*6*) Ibid. **7**, 91(1923); **8**, 602(1925); **16**, 298(1933).
(*7*) Ibid. **24**, 119(1941); **25**, 365(1942).
(*8*) Ibid. **14**, 416(1931); **16**, 298(1933).
(*9*) Ibid. **16**, 298(1933); **22**, 302(1939).
(*10*) Ibid. **26**, 352(1943).
(*11*) Ind. Eng. Chem., Anal. Ed. **7**, 38(1935).
(*12*) J. Assoc. Offic. Agr. Chemists **41**, 274(1958).
(*13*) Ibid. **14**, 397(1931); **16**, 305(1933); **22**, 302(1939).
(*14*) Ibid. **15**, 331(1932); **16**, 293(1933).
(*15*) Ibid. **31**, 498(1948); **32**, 506(1949).
(*16*) Ibid. **10**, 411(1927); **15**, 341(1932); **20**, 155(1937); **21**, 179(1938); **30**, 50(1947); **31**, 498(1948); **32**, 53(1949).
(*17*) Ibid. **27**, 204(1944); **31**, 134(1948).
(*18*) Ibid. **31**, 134, 734(1948).
(*19*) Ibid. **32**, 787(1949).
(*20*) Ibid. **30**, 575(1947); **31**, 739(1948); **32**, 731(1949).
(*21*) Ibid. **42**, 289(1959).

17. Enzymes

Catalase in Vegetables (*1*)—First Action

17.001 REAGENTS

Relatively Stable Reagents

(**a**) *Phosphate buffer soln.*—0.1*M*, pH 7.0. Dissolve 15.22 g $K_2HPO_4.3H_2O$ and 4.54 g KH_2PO_4 in H_2O and dil. to 1 L (pH should be 6.8–7.1).

(**b**) *Sulfuric acid containing molybdate.*—2*N*. Add 55 ml H_2SO_4 to ca 800 ml H_2O, cool, add 0.1 g finely ground $(NH_4)_6Mo_7O_{24}.4H_2O$, agitate until completely dissolved, and dil. to 1 L with H_2O.

(**c**) *Sodium thiosulfate (ca 0.01N) in 10% potassium iodide soln.*—Dissolve 100 g KI, 2.50 g $Na_2S_2O_3.5H_2O$, and ca 1 g Na_2CO_3 in 500 ml H_2O and dil. to 1 L. (Need not be stdzd.)

(**d**) *Iodine soln.*—0.01*N*. Dissolve 1.27 g I and 2 g KI in 10 ml H_2O and dil. to 1 L. Stdze against std $Na_2S_2O_3$ soln, **42.035.** (Avoid exposure to excessive light even while in buret.)

Relatively Unstable Reagents

(**e**) *Hydrogen peroxide.*—0.1*N*. Dil. 0.58 ml 30% H_2O_2 to 100 ml with cold H_2O. Keep in refrigerator or ice bath when not in use. *Prep. daily.*

(**f**) *20% soln of dextrose in buffer soln.*—Dissolve 20 g dextrose in 100 ml of the 0.1*M* phosphate buffer soln, (**a**). Keep refrigerated and prep. weekly.

(**g**) *Starch indicator.*—Add 1.0 g sol. starch to 100 ml cold H_2O, stir thoroly, and heat to boiling. Prep. biweekly.

17.002 PREPARATION OF EXTRACT

To avoid contamination with oxides of heavy metals, handle samples *only* with nonmetallic or stainless steel spatulas.

(**a**) *Undried or frozen vegetables.*—Comminute 50 g portions of undried or frozen vegetables in high speed blender 3 min. with ca 1 g $CaCO_3$ and enough H_2O to make total vol. 200 ml. Remove larger particles by filtering thru 6″ gauze-backed cotton milk filter. Assay filtrate within 30 min.

(**b**) *Dried vegetables.*—Rehydrate 5 g sample and ext. as in (**a**).

17.003 DETERMINATION

(**a**) *To demonstrate presence or absence of catalase.*—To 10 ml ext. (or less, depending on activity) add H_2O to total vol. of 43 ml and 5 ml of the buffered dextrose soln. Mix, and add 2 ml of the 0.1*N* H_2O_2. *Immediately* after addn of the H_2O_2, thoroly mix and quickly remove "zero-time" aliquot of completed reaction mixt. with rapid-flow pipet and blow it into 125 ml erlenmeyer contg 10 ml of the H_2SO_4-molybdate soln. Count "zero time" from time that delivery of aliquot from pipet is started. Remove 10 ml aliquots at 5 and 10 min. (Temp. of reaction mixt. must remain at <20°.) At any time within 1 hr add, to each flask, 5 of the $Na_2S_2O_3$–KI soln and mix. Let stand 3–5 min.; then titr. excess $Na_2S_2O_3$ with the 0.01*N* I, using ca 10 drops of the starch indicator.

Perform blank in exactly same manner, except to add H_2O instead of H_2O_2, and do not remove 5 and 10 min. aliquots. Differences between titer value of blank and of values obtained in presence of H_2O_2 are the I soln equivs of the H_2O_2 present at the respective times. "Zero-time" titer value should be 0.5–2 ml of the I soln; differences between blank-titer value and "zero-time" titer value should be 3.0–4.5 ml 0.01*N* I. Difference of <3.0 ml (*i.e.*, I titer values >2 ml) indicates that the H_2O_2 was too weak or that catalase activity was so high that large quantity of H_2O_2 was decomposed before "zero-time" aliquot was removed. In latter case differences corresponding to 5 and 10 min. may be nearly 0, even when large quantities of catalase are present. In such case catalase content of ext. can be detd by method (**b**) if desired. If 10 ml aliquots of ext. are used, blank-titer value is 4–6 ml, and "zero-time" titer value is <2 ml, catalase is indicated to be absent (within experimental error) when titer values for 5 min. and 10 min. do not differ from "zero-time" titer value by >0.20 ml and 0.40 ml, resp.

(**b**) *To determine accurately catalase activity of sample in terms of K_f.*—"Katalase Fähigkeit," K_f, is k, first order reaction constant (log base 10) detd at 0°, divided by g sample/50 ml reaction mixt. That is, $K_f = k/\text{g}$, and is therefore expression of catalase activity of the prepn. Pure catalase has K_f of 40,000–60,000, depending on source. If K_f is to be detd, make assay exactly as in (**a**) except to maintain reaction mixt. at 0°. It is generally desirable to obtain 15 min. titer value in addn to those described in (**a**). If sample is enzyme prepn of high activity rather than a vegetable, dissolve suitable quantity in dil. buffer, pH 6.5–7.5, preferably contg 2% dextrose.

17.004 CALCULATION OF K_f

Value of K_f for 0–5 min. period and 5–10 min. period should check at 0°, but at higher temps

K_f (5–10 min.) may be $<K_f$ (0–5 min.), because catalase is inactivated by H_2O_2 at significant rate at the higher temps. K_f is given by following formula:

$$K_f = \frac{\left(\frac{1}{t_b - t_a}\right) \log \left(\frac{\text{blank titer} - \text{titer at } t_a}{\text{blank titer} - \text{titer at } t_b}\right)}{\text{g sample/50 ml reaction mixt.}},$$

where t_a and t_b are initial and final times for 2 titer values under consideration. For exts of undried vegetables prepd as in **17.002**, the g sample in reaction mixt. is obtained with enough accuracy by multiplying ml ext. used by 0.25. For most nearly accurate results enzyme concn should be adjusted so that difference between blank titrn and the t_0 titrn is 3–4 ml, and difference between blank titrn and the t_{10} titrn is 0.5–1 ml of the I soln.

Diastatic Activity of Flour (*2*)—Official

17.005 REAGENT

Buffer soln.—Dil 3 ml HOAc and 4.1 g *anhyd.* NaOAc to 1 L with H_2O; pH of this soln is 4.6–4.8.

17.006 DETERMINATION

(Total maltose after diastasis 1 hr)

Place 5 g flour and teaspoonful ignited *quartz sand* in 100 or 125 ml erlenmeyer, and mix by rotating flask. Add 46 ml of the buffer soln, and again mix by rotating flask until all flour is suspended. Bring flask and all ingredients *individually* to 30° before mixing. Digest 1 hr at 30°, preferably in thermostat-controlled H_2O bath, shaking flask (by rotation) every 15 min. After 1 hr add 2 ml H_2SO_4 ($3.58 \pm 0.05N$, ca $1+9$), and mix thoroly. Add 2 ml *12% $Na_2WO_4.2H_2O$ soln*, mix, and let stand 1–2 min. Filter thru paper (Whatman No. 4 or equiv.), discarding first 8 or 10 drops. Proceed as in **13.029(b)**.

Foregoing specifications may be used with all ordinary flours whose values for mg maltose produced by 10 g flour in 1 hr seldom, if ever, exceed 350. For material giving higher values, such as products from malted or sprouted grain, use smaller portions of ext., *i.e.*, 1, 2, or 3 ml instead of 5 ml. In such cases, however, add enough H_2O to make up difference, and use appropriate factor to convert results into mg maltose/10 g flour. If material in test tubes is colorless instead of yellow after treatment in boiling H_2O bath and gives no blue color upon addn of KI, it is apparent that there was more than enough maltose to reduce all the $K_3Fe(CN)_6$, and detn must be repeated with smaller quantity of ext.

17.007 BLANK DETERMINATION

Blank detn to indicate quantity of reducing sugar originally present in the flour—value for which presumably should be deducted from total maltose value after 1 hr's diastasis—has been generally regarded as essential step in estimation of flour diastatic activity. This operation ordinarily is unnecessary when dealing with flour milled from *sound* wheat, because quantity of reducing sugars originally present as such is so small and so nearly constant that it may be disregarded for all practical purposes. Blank detn may therefore conveniently be omitted in routine testing. It need be used only when there is occasion to doubt soundness of the wheat, or where there is known to have been appreciable quantity of frosted, sprouted, heat-damaged, or otherwise unsound kernels in wheat from which the flour was milled.

If blank detn is desired, proceed as in **13.029(a)**.

17.008 Diastatic Power of Malt and Malt Sugars—*See* **10.079** and **10.115**

17.009 Phosphatase, Residual, in Dairy Products—*See* **15.047–15.056, 15.076–15.078, 15.137, 15.160–15.163,** and **15.182**

Proteolytic Activity of Flour and Malted Wheat Flour (*3*)—Official

(Applicable to slightly active materials such as patent flour or to dild exts of active proteolytic prepns)

17.010 REAGENTS

(**a**) *Buffer stock soln.*—Dil. 120 ml HOAc and 164 g anhyd. NaOAc to 1 L with H_2O. Dil. with 20 vols H_2O before using (pH 4.7).

(**b**) *Bacto-hemoglobin substrate.*—Obtainable from Difco Laboratories, Detroit, Mich.

(**c**) *Trichloroacetic acid soln.*—Dissolve 180 g trichloroacetic acid in 320 ml H_2O.

17.011 DETERMINATION

(**a**) *Preparation of enzyme solns.*—For slightly active materials such as flour, weigh as much as 10 g directly into digestion flasks. For active enzyme prepns, prep. ext. or suspension in dild buffer, **17.010(a)** immediately preceding digestion. (Quantity of ext. or dilns thereof used in digestion mixt. may vary up to 2 ml; appropriate activation technics may be applied to enzyme exts.)

(**b**) *Digestion procedure.*—Weigh 2.50 g (H_2O-free basis) Bacto-hemoglobin into each of two 125 ml erlenmeyers, add ca 5 g or 1 teaspoon fine pumice and flour sample, (**a**), to each flask, and agitate mixt. by rotation until flour and substrate are intimately mixed. Then add, to each flask, 50 ml of the dild buffer soln, previously warmed to $40 \pm 0.1°$ in thermostat-controlled bath, and agitate mixt. to suspend uniformly. Place tightly stoppered flasks in the 40° bath and agitate either continuously or at 1 hr intervals.

Add 10 ml portion of trichloroacetic acid soln (**c**)

to one flask after 15 min. digestion and to second flask after 5.25 hr digestion. Shake each flask, using 25 vigorous horizontal movements, and keep flasks in bath at 40° exactly 30 min. Centrifuge suspension 5 min. at 1800 rpm and filter. (Some materials such as flour may remain turbid after final filtration; clear by boiling centrifuged digestion mixt. few sec. before final filtration. Replace liquid lost thru evapn by adding H_2O.) Pipet duplicate 10 ml aliquots directly into Kjeldahl flasks and det. sol. N.

Follow essentially same procedure in detg enzyme activity of an ext. In place of the solid material, use total of 2 ml ext. or ext. plus dild buffer soln. After zero time and 5 hr digestion periods, add to each flask 10 ml aliquot of trichloroacetic acid soln (**c**). Mix contents thoroly, keep in H_2O bath exactly 30 min., and filter without centrifuging. Analyze 10 ml aliquots for sol. N.

(**c**) *Detn of soluble nitrogen*.—Proceed as in **2.036**. Use definite vol. H_2O (350 ml) to dil. cooled digest and add in such way as to wash down all trichloroacetic acid that has condensed in neck of flask during digestion. Also add the NaOH soln, **2.034(f)** (1.5 times usual quantity) so as to rinse neck of flask. After distn, back-titr. the unneutralized std acid with 0.0714*N* NaOH.

(**d**) *Expression of proteolytic activity*.—Proteolytic activity is measured by difference in back-titrn vols for 15 min. or zero time digestions and corresponding long-time digestion, expressed in ml 0.0714*N* NaOH. Transform proteolytic activity detd for 10 ml aliquot to 3/2 power. Multiply this value by 6 (total final vol. of digest/10 ml aliquot) and by 1000/mg enzyme source. This value is activity expressed in hemoglobin units (HU)/g enzyme prepn (*4*).

NOTES: (1) Careful washing down of trichloroacetic acid from neck of digestion flasks is mandatory. If not neutralized, trichloroacetic acid steam distills.

(2) More reproducible results will be obtained if Kjeldahl detns are completed without delay between digestion and distn.

(3) If other than 10 ml aliquots are analyzed for sol. N, convert results to 10 ml aliquot basis before transforming to 3/2 power. If titrn difference, using 10 ml aliquot, is >10 ml 0.0714*N* NaOH, reanalyze, using smaller quantity of enzyme. For most precise results, titrn difference should be 4.0–6.0 ml 0.0714*N* NaOH.

(4) For each lot of hemoglobin, adjust pH of stock buffer, if necessary, so that pH of mixt. of 50 ml dild buffer, 2.5 g hemoglobin, and ca 5 g pumice will be 4.70 ± 0.05. This pH for buffer substrate mixt. is critical for accuracy of method.

Proteolytic Activity of Papain (*5*)
First Action

17.012 REAGENTS

(**a**) *Casein soln*.—Make 6% soln of Hammarsten casein (obtainable from Nutritional Biochemicals Corp., 21010 Miles Ave., Cleveland 28, Ohio) by rubbing 60 g with little H_2O in mortar and gradually adding 60 ml 1*N* NaOH and H_2O until vol. totals 1 L. Heat viscous soln 30 min. in boiling H_2O bath, cool, and filter thru glass wool if necessary.

(**b**) *Citrate buffer soln*.—Prep. 0.2*M* monosodium citrate soln by partial neutralization of citric acid with NaOH.

(**c**) *Titrating soln*.—0.1*N* alc. KOH.

(**d**) *Indicator*.—1% alc. thymolphthalein soln.

17.013 PREPARATION OF SAMPLE

(**a**) *Unactivated*.—If enzyme prepn is solid, grind to smooth paste in small mortar with little freshly boiled, cold H_2O. Then suspend in cold boiled H_2O in proportion of 10 mg original prepn/ml H_2O. After 5–10 min. centrifuge suspension and discard sediment.

(**b**) *Activated*.—Proceed as in (**a**), but use half-satd H_2S-H_2O instead of boiled H_2O. After centrifuging, incubate enzyme soln 1 hr at 40° to complete activation.

17.014 DETERMINATION

Place 10 ml of the casein soln and small charge of 4 mm diam. glass beads in each of several 125 ml g-s. bottles, and bring bottles and contents to 40°. Add desired vol. of the prepd enzyme soln, but do not use >4 ml. If this quantity is insufficient (*see* **17.015**), prep. more concd soln of the enzyme. Add immediately exactly 3 ml of the buffer soln (pH of system should then be 5.0 ± 0.1). Shake bottle vigorously few sec. and place in constant temp. H_2O bath at 40°.

Incubate mixt. 20 min. at 40°, counting time from addn of buffer. Add 1 ml of the indicator and begin titrg with the alc. KOH soln. As soon as deep blue appears, shake bottle until color is discharged or ppt is completely dissolved. (It is usually best to add alkali in ca 0.5 ml portions.) When all pptd casein dissolves, transfer contents of bottle to 400–500 ml flask and rinse bottle 2 or 3 times with alcohol, using total of 25 ml. Add enough of the KOH soln to restore blue color; then add 175 ml boiling alcohol. Carefully add more KOH soln until pale but distinct blue persists in soln.

Make control titrn exactly as described, but do it immediately after addn of the buffer, without any incubation time. Difference between titrn of undigested sample and that of digested sample is measure of proteolytic activity of the enzyme.

17.015 CALCULATION OF PROTEINASE UNIT

For smaller quantities of enzyme, extent of hydrolysis detd by above titrn is straight line

function of quantities of papain used. For accurate work det. this straight line by making several titrns with different quantities of enzyme. If quantities of papain used are too large, straight-line relationship no longer holds; if they are too small, detn is inaccurate. Quantities of enzymes giving titrn differences of 0.6–1.2 ml 0.1*N* KOH are recommended.

Unit of papain may be considered to be quantity of enzyme that produces, under conditions outlined, titrn difference of 1 ml 0.1*N* KOH, detd either graphically or arithmetically. Value of original prepn is then expressed in units/mg, or as mg papain prepn necessary to make one unit.

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(*3*) J. Assoc. Offic. Agr. Chemists **30,** 659 (1947); **32,** 261(1949); **43,** 560(1960).

(*4*) Arch. Biochem. **32,** 200(1951).

(*5*) J. Assoc. Offic. Agr. Chemists **18,** 140 (1935); **19,** 373(1936); **21,** 97(1938).

18. Fish and Other Marine Products

18.001 Preliminary Treatment and Preparation of Sample (*1*)—Procedure

To prevent loss of H_2O during prepn and subsequent handling, use as large samples as practicable. Keep ground material in container with airtight cover. Begin all detns as soon as practicable. If any delay occurs, chill sample to inhibit decomposition. Prep. samples for analysis as follows:

(**a**) *Fresh fish.*—Clean, scale, and eviscerate large fish in usual way. In case of small fish (6″ long or less), use 5–10 whole fish, including heads if desired. In case of large fish, cut from each of at least 3 fish, 3 transverse slices, 1″ thick: one slice from just back of pectoral fins, one slice halfway between first slice and vent, and one slice just back of vent. Skin and bones may be sepd if desired. For fat detns, include skin since many fish store large quantities of fat directly beneath skin.

Pass sample rapidly thru meat chopper 3 times. Remove unground material from chopper after each grinding and mix thoroly with ground material. Meat chopper should have holes as small as practicable ($\frac{1}{16}$–$\frac{1}{8}$″ diam.) and should not leak around handle end. As alternative procedure for soft fish, high speed blender may be used. Grind several min., stopping blender frequently to scrape down sides of cup.

(**b**) *Canned fish and other canned marine products.*—Macerate entire contents of container in blender or pass thru meat chopper 3 times as in (**a**).

(**c**) *Canned marine products packed in oil.*—Drain 2 min. on No. 8 sieve. Prep. solid portion as in (**b**). Oil and brine may be analyzed separately, if desired, or reincorporated with solids.

(**d**) *Fish packed in salt or brine.*—Drain brine and rinse off adhering salt crystals with satd NaCl soln. Drain again 2 min. and proceed as in (**a**).

(**e**) *Dried smoked or dried salt fish.*—Cut large samples into small pieces, mix, and quarter down to ca $\frac{1}{4}$ lb. Cut, shred, grind, or otherwise comminute the $\frac{1}{4}$ lb sample as finely as possible so that reasonably representative samples may be weighed for analysis after thoro mixing. (Duplicate or triplicate detns may be necessary to establish uniformity of sample.)

(**f**) *Shellfish other than oysters, clams, and scallops.*—If sample is received in shell, wash as in (**g**) and sep. edible portions in usual way. Prep. edible portion for analysis in as (**b**).

(**g**) *Shell oysters, shell clams, and scallops.*—Wash shells in potable H_2O to remove all loose silt and dirt, and drain well. Shuck enough oysters or clams into clean dry container to yield at least 1 pint drained meats. Transfer shellfish meats to skimmer, **18.003**, pick out pieces of shell, drain 2 min. on skimmer, and proceed as in (**h**) or (**i**).

(**h**) *Shucked clams or scallops.*—Prep. as in (**b**).

(**i**) *Shucked oysters* (*2*).—Grind meats, including liquid, 1–2 min. in high speed blender.

18.002 Volume Determination (*1*)—Official

(Shucked oysters, clams, or scallops)

Fluff entire contents of commercial container, or container in which sample is received (1 gallon or less) by pouring into std measuring vessel thru distance of at least 1 ft, then pouring back into container from same height, and again pouring into measuring vessel. Use metal funnel (stainless steel preferable) 8–10″ diam. at top, with stem 3″ diam. and ca 3″ long, to facilitate pouring from one vessel to another. Measures are straight-side, cylindrical, made of metal (stainless steel preferable), holding exactly 1 gallon or 1 quart, resp., and having smooth rims. Plane of rim must be level when measure is standing on level surface. Diam. of top of gallon measure is 4.25–5.25″, and that of quart measure is 3.25–3.5″. Calibrate with std glass measures, and for estimating vols less than level full, use graduated mechanic's depth gauge to measure distance from rim to surface of contents. Tabulate depth gauge readings against vols or % shortages as desired for each measuring vessel. Measure head space with depth gauge and det. vol. For 1 pint or smaller containers, calibrated glass cylinders may be used.

Drained Liquid (*3*)—Official

(Shucked oysters)

18.003 APPARATUS

Skimmer or strainer.—Flat-bottom metal pan or tray with ca 2″ sides, with area of not <300 sq. in. for each gallon of oysters to be poured on tray, and with perforations 0.25″ diam. and 1.25″ apart in square pattern, or perforations of equiv. area and distribution. Support skimmer over

slightly larger solid tray so that liquid drains into solid tray.

18.004 DETERMINATION

Weigh tared container with shellfish meats, transfer contents to skimmer, and quickly distribute meats evenly over draining surface with min. of handling. Drain 2 min., return meats to container, and reweigh. Calc. loss of wt as % drained liquid. Make detns at $7 \pm 1°$ ($45 \pm 2°F$). If further analysis is desired, proceed as in **18.001**(i).

Total Solids

18.005 *For All Marine Products Except Raw Oysters (4)—Official*

Cut into short lengths ca 2 g asbestos fibers of type used in prepg gooches. Place cut fibers and glass stirring rod ca 8 cm long with flat end into flat-bottom metal weighing dish, ca 9 cm diam., with cover. Dry dish, asbestos, and rod in oven 1 hr at 100°, cool, and weigh. Weigh quickly into dish, to nearest mg, 9.5–10.5 g prepd sample. Add 20 ml H_2O and mix sample thoroly with asbestos. Support end of rod on edge of dish and evap. just to dryness on steam bath, stirring once while still moist. Drop rod into dish and heat 4 hr in oven at 100°, or in preheated force-draft oven set for full draft, 1 hr at 100°. Cover dish, cool in desiccator, and weigh promptly.

18.006 *For Raw Oysters Only—First Action*

Weigh quickly, to nearest mg, 9.5–10.5 g prepd sample into weighed, flat-bottom metal dish ca 9 cm diam. Spread sample evenly over bottom of dish.

(**a**) (*5*) Evap. just to dryness on steam bath and dry 3 hr in oven at 100°; or—

(**b**) (*6*) Insert directly into preheated force-draft oven set at full draft and dry 1.5 hr at 100°.

Cool in desiccator and weigh promptly.

18.007 **Ash (*7*)—Official**

Dry sample representing ca 2 dry material and proceed as in **29.012** or **29.013**, using temp. not $>550°$. If material contains large quantity of fat, make preliminary ashing at low enough temp. to allow smoking off of fat without burning.

Salt (Chlorine as Sodium Chloride) (*8*)—Official

18.008 REAGENTS

(**a**) *Silver nitrate soln.*—$0.1N$. Prep. as in **42.025** and stdze against $0.1N$ NaCl contg 5.845 g of pure dry NaCl/L.

(**b**) *Ammonium thiocyanate soln.*—$0.1N$. Prep. as in **42.028**(b) and stdze against the $0.1N$ $AgNO_3$.

(**c**) *Ferric indicator.*—Satd soln of $FeNH_4(SO_4)_2.12H_2O$.

18.009 DETERMINATION

(**a**) *Shellfish meats*—Weigh 10 g meats, liquid, or mixed meats and liquid, into 250 ml erlenmeyer or beaker.

(**b**) *Other fish products.*—Use suitable size sample, depending on NaCl content.

Add known vol. of the $AgNO_3$ soln, more than enough to ppt all the Cl as AgCl, and then add 20 ml HNO_3. Boil gently on hot plate or sand bath until all solids except AgCl dissolve (usually 15 min.). Cool, add 50 ml H_2O and 5 ml of the indicator, and titr. with the NH_4SCN soln until permanent light brown color appears. Subtract ml $0.1N$ NH_4SCN used from ml $0.1N$ $AgNO_3$ added and calc. difference to NaCl. With 10 g sample each ml $0.1N$ $AgNO_3 = 0.058\%$ NaCl.

18.010 **Total Nitrogen (*9*)—Official—** *See* **2.036**

Crude Fat

By Acid Hydrolysis (10)—Official

18.011 PREPARATION OF SAMPLE

Prep. sample according to type of pack as in **18.001** and keep ground material in sealed jar. If jar has been chilled, let sample come to room temp. and shake jar so that any sepd liquid is absorbed by fish. Open jar and stir contents with spatula, thoroly scraping sides and lid so as to incorporate any sepd liquid or fat.

18.012 DETERMINATION

Weigh into 50 ml beaker 8 g well-mixed sample and add 2 ml HCl. Using stirring rod with extra large flat end, break up coagulated lumps until mixt. is homogeneous. Add addnl 6 ml HCl, mix, cover with watch glass, and heat on steam bath 90 min., stirring occasionally with rod. Cool soln and transfer to Mojonnier fat-extn tube. Rinse beaker and rod with 7 ml alcohol, add to extn tube, and mix. Rinse beaker and rod with 25 ml ether, added in 3 portions; add rinsings to extn tube, stopper tube with cork or stopper of synthetic rubber unaffected by usual fat solvents, and shake vigorously 1 min. Add 25 ml petr. ether (b. p. $<60°$) to extn tube and repeat vigorous shaking. Centrifuge Mojonnier flask 20 min. at ca 600 rpm and proceed as in **13.019**, beginning "Draw off as much as possible of ether-fat soln . . ."

Drying to constant wt takes ca 40 min. for fish. Long heating periods may increase wt of fat. If centrifuge is not available, extn can generally be made by letting Mojonnier flask stand until upper liquid is practically clear, then swirling

flask and again letting stand until clear. If troublesome emulsion forms, pour off from Mojonnier flask as much of ether-fat soln as possible after letting flask stand, add 1–2 ml alcohol to Mojonnier flask, swirl, and again let mixt. sep.

Rapid Modified Babcock Method (11)—First Action

(For canned fish)

18.013 DETERMINATION

Weigh 9 g ground and mixed sample into Paley-type Babcock cheese bottle (Kimble Glass No. 508, 20% size), stopper, and add ca 30 ml of reagent prepd by mixing equal vols HOAc and 70–72% $HClO_4$. Place in boiling H_2O bath (stainless steel beaker preferred), swirling occasionally until digestion is complete (ca 20 min.). Remove from bath, add reagent until fat is well up in calibrated neck of bottle, centrifuge 2 min. at ca 600 rpm, and read % fat with dividers. If fat falls below calibration, add more reagent, centrifuge 1 min., and read again.

18.014 Fat in Fish Meal—First Action— *See* **22.037**

Volatile Fatty Acids (*12*)—Official

18.015 APPARATUS

(**a**) *Steam distillation assembly.*—Fig. 31. Assembly consists of boiler flask (3 L) giving steam at constant rate so as to produce constant rate of distn, distn flask, condenser, and 200 ml vol. flasks as receivers. Std distn flask with side arm (ca 9 mm o. d.) attached near center of neck, and with steam inlet tube (ca 10 mm o. d.), is satisfactory. Heating coil of steam generator is made by winding 5 feet 28 gauge Chromel wire (or equiv.) around hollow pipe ca 0.25″ diam. and heating red hot to detemper wire. Leads into boiler flask are brass, Cu, or other non-ferrous metal ca 3/32″ diam.

Any similar distn assembly may be used if it is of capacity to handle vols specified in method and gives 57 ± 2% recovery of acetic acid on distn.

(**b**) *Chromatographic tube.*—Approx. 15 × 250 mm.

(**c**) *Source of air pressure or compressed N gas equipped with pressure regulator.*—If such source is not available, following system serves purpose: Fit 1 L side arm flask with 2 hole rubber stopper. Pass glass manometer tube 70 cm long thru one hole in stopper so that it reaches bottom of flask, and thru other hole pass glass tube ca 8 cm long, whose upper end is connected to top of chromatographic tube by rubber tubing. Connect rubber hand-aspirator bulb to side arm of flask. Fill flask with Hg to depth of 1.5 cm. (Height of Hg column in manometer tube indicates pressure in system; 25 cm is equiv. to ca 5 lbs.) To maintain reservoir pressure when chromatographic tube is disconnected, fit stopcock into line leading from flask to

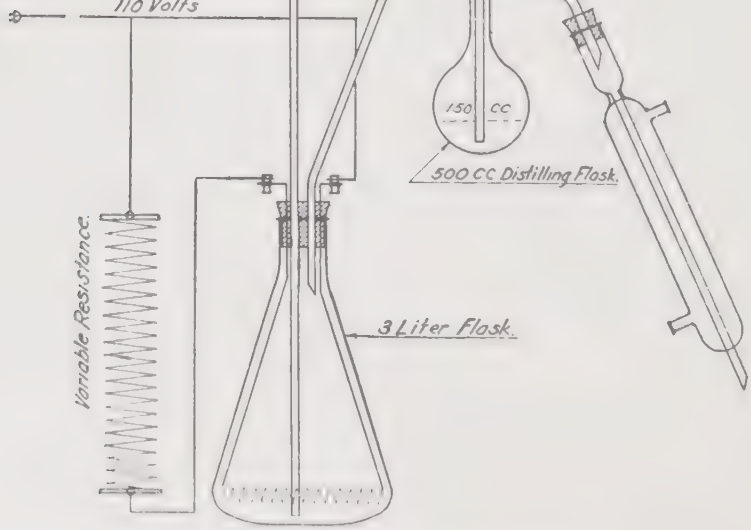

FIG. 31.—STEAM DISTILLATION ASSEMBLY

chromatographic tube; and to prevent valve in hand aspirator from leaking, insert second stopcock between side arm and bulb.

(d) *Rubber bulb.*—5 ml. (Type used on dropping bottles.)

18.016 REAGENTS

(a) *Butanol in chloroform.*—1%. Remove alcohol from USP $CHCl_3$ by washing 3 times with ½ vol. H_2O. Add 10 ml *n*-butanol to 1 L washed $CHCl_3$ in separator, shake vigorously, add 25 ml H_2O, and shake again. Let lower layer stand until clear, and drain. Discard aq. layer.

(b) *Butanol in chloroform.*—10%. To 900 ml USP $CHCl_3$ (*not* previously washed) in separator add 100 ml *n*-butanol, shake vigorously, add 25 ml H_2O, and shake again. Let $CHCl_3$ stand until clear, and drain. Discard aq. layer.

(c) *Alphamine Red R indicator.*—Dissolve 50 mg mono-NH_4 salt 3-(4-anilino-1-naphthylazo)-2,7-naphthalene disulfonic acid (Eastman No. 6410) in 25 ml H_2O. (Red color produced by 1 drop indicator in 20 ml H_2O must be changed to violet by 1 drop 0.01N HCl.)

(d) *Cresol red indicator.*—Dissolve 50 mg *o*-cresolsulfonphthalein in 20 ml alcohol, add 1.3 ml 0.1N NaOH, and dil. to 50 ml with H_2O. Use 2 drops for each 25 ml aq. soln.

(e) *Barium hydroxide soln.*—0.01N. (Store in paraffin-lined bottle and protect from CO_2 of atmosphere with soda-lime or Ascarite; dispense from 10 ml buret.) (0.01N NaOH may be used instead of $Ba(OH)_2$ except in titrn of Soln *B*, **18.019.** NaOH soln must be used in titrn of Soln *A*, **18.019,** since Na salts are required for chromatography.)

(f) *Sodium acetate-sodium chloride soln.*—Dissolve 12 g NaCl and 25 g $NaOAc.3H_2O$ in H_2O and dil. to 500 ml.

(g) *Silicic acid.*—Reagent grade "100-mesh" powder, suitable for chromatography (Mallinckrodt Chemical Co. No. 2847 or equiv.).

18.017 STANDARDIZATION OF DISTILLATION APPARATUS

Place app., **18.015**(a), in laboratory so that it is free from drafts and sudden changes in temp. Make mark on boiler flask at 1.5 L level, fill to this mark with H_2O, heat to boiling, and boil several min. before starting distn. Transfer 150 ml H_2O to distn flask, add 1 drop H_2SO_4 (1+1), connect condenser, insert steam inlet tube into distn flask, and bring contents of flask to incipient boiling with burner. Connect steam inlet tube with steam supply from boiler, and steam distill. Regulate rate of evolution of steam and height of small flame of burner under distn flask so that vol. of liquid in distn flask is kept constant at 150 ml and distillate collects at rate of 200 ml/hr. (Period of collection may vary 5 min. for 200 ml of distillate. The 150 ml vol. in distn flask should remain constant within ±10 ml. Boiling may be stopped to permit test of constancy of 150 ml vol. by momentarily interrupting steam supply. Few trials will show conditions necessary to maintain constant vol. in distn flask and constant rate of distn.) Det. blank on 2 successive 200 ml portions of distillate by titrg with 0.01N alkali (phthln) in CO_2-free atmosphere.

Transfer 50 ml ca 0.1N HOAc (concn must be accurately known) to distn flask; add 1 drop H_2SO_4 (1+1) (avoid contact with neck of flask) and 100 ml H_2O. Collect 200 ml distillate and titr. with 0.1N alkali. Correct for titrn blanks and compute % acid distd. Distn technic and app. are satisfactory when recovery is 57±2%. App. so adjusted gives distn rates on propionic and butyric acids of 81% and 92% (±2%), resp. (*13*), on 200 ml distillate.

18.018 PREPARATION OF SOLUTION

Comminute sample (include entire contents of canned products) by passing 3 times thru food chopper, mixing after each grinding. Weigh 50 g comminuted material into tared 500 ml widemouth erlenmeyer, add ca 150 ml H_2O, stopper flask, and shake vigorously ca 1 min. to effect thoro suspension of material. Add 25 ml 1N H_2SO_4, mix, ppt proteins with *20% phosphotungstic acid soln* (40 ml is usually enough), make to 300 g with H_2O, shake vigorously ca 1 min., and filter thru 24 cm rapid folded paper.

18.019 DISTILLATION AND COMPUTATION OF VOLATILE ACID NUMBER

Pipet 150 ml prepd soln into distn flask of app. and make acid to *Congo red paper* with H_2SO_4 (1+1). Steam distill as in **18.017.** Collect 200 ml distillate, titr. with 0.01N NaOH, and designate as *A*. Collect second 200 ml portion distillate, titr. with the 0.01N $Ba(OH)_2$ soln, and designate as *B*. To calc. volatile acid number multiply titrn obtained on distillate *A*, corrected for blank, by 4.

DETERMINATION OF INDIVIDUAL VOLATILE FATTY ACIDS

18.020 *Formic Acid*

Add 2 drops satd $Ba(OH)_2$ soln to distillate *B*, **18.019,** and evap. to dryness on steam bath. Add ca 5 ml H_2O to residue and 1 ml more of 1N HCl than necessary to liberate volatile acids. Filter thru small paper into 125 ml erlenmeyer with ₮ joint, and wash paper with H_2O in such manner that total filtrate equals 30–40 ml. Add 10 ml of the NaOAc-NaCl soln and 10 ml *5% $HgCl_2$ soln.* Connect flask with ₮ air condenser and place on steam bath 2.5 hr.

With suction thru glass siphon attached to funnel by rubber stopper, transfer ppt of Hg_2Cl_2 to previously weighed ACS-type microfunnel having coarse glass disk provided with mat of asbestos ca 2 mm thick. Rinse flask with H_2O followed by alcohol. Dry 30 min. at 100°, cool, and weigh. Weigh funnel with another funnel, prepd with asbestos and treated similarly as one contg ppt, as counterpoise.

Wt Hg_2Cl_2 (mg) $\times 0.0975 =$ mg formic acid in distillate. To calc. total formic acid originally present in aliquot of sample in distn flask before distn, divide mg formic acid found by 0.24 (fraction formic acid distd in second 200 ml distillate) and multiply by 4 to obtain formic acid in 100 g sample being analyzed.

18.021 *Chromatographic Separation of C_2 to C_4 Saturated Fatty Acids*

(**a**) *Preparation of partition column.*—To 5 g silicic acid in mortar add 1 ml of the Alphamine Red R indicator soln and enough $1N$ NH_4OH to give alk. color of the indicator (1 drop is usually enough). Add max. quantity of H_2O that the silicic acid will hold without becoming sticky or agglomerating in the butanol-$CHCl_3$ soln. (This quantity must be detd for each batch of silicic acid and usually varies from 50–75% of wt of silicic acid.) Mix thoroly with pestle until homogeneous. Add few ml of the 1% butanol in $CHCl_3$, mix to form paste, and then add enough solvent to form slurry that pours readily. Pour this slurry into chromatographic tube contg small cotton plug in neck of constricted end. To avoid air pockets tilt tube slightly while pouring. If air bubbles form while pouring, eliminate by stirring suspension in tube with long glass rod.

Clamp tube vertically in ring stand. In top insert 1 hole rubber stopper fitted with glass tube bent to 90° angle and held in place by Bunsen clamp against pressure to be exerted. Connect bent glass tube to pressure source, **18.015(c)**. Adjust pressure to 5–10 lbs/sq. in., so that excess solvent is forced thru column dropwise.

During removal of excess solvent the gel packs down. As column packs down, particles of gel adhere to wall of the tube, but eventually gel leaves wall of tube relatively clean. This is point of optimum density for column, and column is ready for use. Do not let column dry below surface of the gel, as such drying or "cracking" renders column useless. If column cracks before acids are added, gel can be extruded from tube, reslurried with solvent, and again poured into tube. If gel is not packed evenly or if air pockets are present, jagged fronts may occur where soln passes such points.

(**b**) *Test of silicic acid for suitability and standardization of column.*—(1) *Preparation of known acid mixt.*—Pipet 1 ml of each of following acids into sep. 50 ml vol. flasks: Formic, acetic, propionic, butyric, and valeric. Dil. to vol. with H_2O, and mix to prep. stock solns of these acids. Pipet 10 ml of each stock soln into a 125 ml erlenmeyer to prep. acid mixt. to be used for stdzn. Keep flask tightly stoppered.

Pipet 1 ml of each stock soln, except formic acid, into sep. erlenmeyers, dil. to ca 15 ml with boiled H_2O, and titr. with the $0.01N$ alkali, using cresol red indicator. End point is reached when soln assumes pink color that persists ca 45 sec. During titrn bubble stream of CO_2-free air or N thru soln. (CO_2-free air can be prepd by bubbling air thru layer of 20% NaOH soln at least 8 cm deep. Trap or cotton filter plug between wash soln and titrn flask prevents contamination by droplets of the NaOH soln. Satisfactory gas washing bottle can be made from 2 L Florence flask fitted with 2 hole rubber stopper.) To det. quantity of each acid present in 1 ml of mixt. divide quantity of each acid in 1 ml of its stock soln by 5.

Pipet 0.1 ml of the acid mixt. into 50 ml beaker, neutralize with $1N$ NaOH, using phthln, and add 1 drop NaOH soln in excess. Evap. to dryness on steam bath.

(2) *Separation technic.*—(Good sepn and yields depend upon transfer of sample to column with quantities of solvent specified.) To dry residue of Na salts add 2 ml of the 1% butanol in $CHCl_3$ soln and 3 drops H_2SO_4 (1+1), and stir with glass rod until all Na salts are converted to free acids (acid to Congo red paper). Add anhyd. Na_2SO_4 in 0.2 g portions until aq. phase is absorbed. Place 50 ml graduated cylinder under prepd column to catch forerun, vol. of which is threshold vol. for valeric acid.

Decant supernatant (or use eyedropper pipet) onto column, pouring it slowly down side of tube in order to keep surface of gel level. Apply pressure until solvent just disappears into gel. Wash beaker with 1 ml of the solvent, pour onto column, and with stirring rod transfer residue in beaker to column. Wash beaker with another 1 ml solvent, transfer to column, wash inside of tube with 1 ml solvent, and apply pressure until solvent just disappears into gel. Fill tube with solvent and apply pressure. Change receiver (graduated cylinder) each time lower edge of a band reaches point 2–5 mm above narrowest portion of constriction of tube. (Acids elute in following order: 1, valeric; 2, butyric; 3, propionic; and 4, acetic.) Record forerun vol., also vol. of solvent required to elute each of remaining acids, except formic. Do not try to elute formic acid band. After propionic acid (third band) reaches constriction of tube, unclamp and pour off remaining solvent in chromatographic tube and fill tube with the 10% butanol in $CHCl_3$.

Det. threshold vol. of butyric acid by adding forerun vol. (valeric threshold vol.) to vol. of solvent required to elute the valeric acid. To obtain threshold vol. of propionic acid add threshold vol. of butyric acid to vol. of solvent required to elute butyric acid. In similar manner obtain threshold vol. of acetic acid.

To compare threshold vols, when different quantities of acids are present, repeat stdzn, using 1 ml of the acid mixt. (ca 4 mg of each acid), but change solvent in chromatographic tube to 10% butanol in $CHCl_3$ when vol. of eluate equiv. to threshold vol. found for ca 0.5 mg propionic acid in first stdzn has been collected.

Transfer eluates to sep. 125 ml erlenmeyers, rinsing each graduated cylinder with three 5 ml portions of H_2O. Add 1 drop of the cresol red indicator soln and titr. with 0.01*N* alkali in CO_2-free atmosphere, as above. However, as end point approaches, stopper flask and shake vigorously to completely ext. acids from solvent phase. Correct titrn of each eluted band for blank as follows: Collect 25 ml of the butanol-$CHCl_3$ mixt. from column before any acids are transferred, add 15 ml boiled H_2O, and titr. as above with 0.01*N* alkali.

If bands are not clearly differentiated or recoveries are <90%, reject the silicic acid. (Addnl stdzn with respect to threshold vol. may be desirable for identification in some instances, (**c**).)

(**c**) *Identification and determination.*—Evap. distillate *A* obtained in **18.019** to small vol., transfer to 50 ml beaker, and proceed exactly as in (**b**)(2). When vol. eluate equiv. to threshold vol. found for 0.5 mg propionic acid in stdzn of column collects, change solvent in tube to the 10% butanol in $CHCl_3$ as above even if no propionic acid is present. Do not change receiver until lower edge of next band reaches point 2–5 mm above narrowest portion of constriction of tube, or until titrn of 5 ml fractions approaches that of the blank.

Identify acids by comparing their threshold vol. with those for ca same quantities and ratios of known acids found in stdzn procedure. Threshold vol. of given quantity of each fatty acid is characteristic and quite reproducible under similar conditions. However, if conditions change, such as by use of different batch of silicic acid or different quantity of same batch, or different quantity of H_2O, threshold vol. for each acid must be redetd. (If present, *iso*-butyric acid is measured as *n*-butyric acid.) For further identification as characteristic salts *see* reference (*14*).

To calc. quantities of acetic, propionic, and butyric acids in terms of ml 0.01*N* alkali in aliquot, divide titrns by 0.57, 0.81, and 0.92, resp.; multiply these values by 2.40, 2.96, and 3.52, resp., to obtain mg of each acid/100 g sample.

Histamine-Like Substances

Biological Method (15)—Official

18.022 APPARATUS

(**a**) *Kymograph.*—Kymograph with horizontal muscle lever arm having friction or gravity writing point.

(**b**) *Muscle bath.*—At least 50 ml capacity, surrounded by 37° constant temp. bath. *See* Fig. 32. May be conveniently filled and emptied thru 3-way stopcock; 1 tube connected to reservoir of Ringer-Locke soln thru bulb or coil immersed in bath; other tube connected to vac. thru suction flask as waste receiver. Bubble air, filtered thru cotton, slowly and continuously around intestine from fine capillary tube.

18.023 REAGENTS

(**a**) *Sodium chloride stock soln.*—180 g/L.

(**b**) *Potassium chloride stock soln.*—42 g/500 ml.

(**c**) *Sodium bicarbonate stock soln.*—15 g/500 ml.

(**d**) *Atropine sulfate stock soln.*—1.0 g/500 ml.

(**e**) *Calcium chloride stock soln.*—24 g anhyd. salt/500 ml.

(**f**) *Ringer-Locke soln.*—NaCl, 0.9%; KCl, 0.042%; $CaCl_2$, 0.024%; $NaHCO_3$, 0.015%; dextrose, 0.1%; and atropine sulfate, 0.001%. Add 100 ml (**a**) and 10 ml (**b**), (**c**), and (**d**) to 2 L vol. flask. Add H_2O to vol. of ca 1800 ml and then 10 ml (**e**) while swirling. Add 2 g anhyd. dextrose before use. Dil. to vol. with H_2O. Keep soln contg dextrose in refrigerator when not in use, discarding when it becomes moldy.

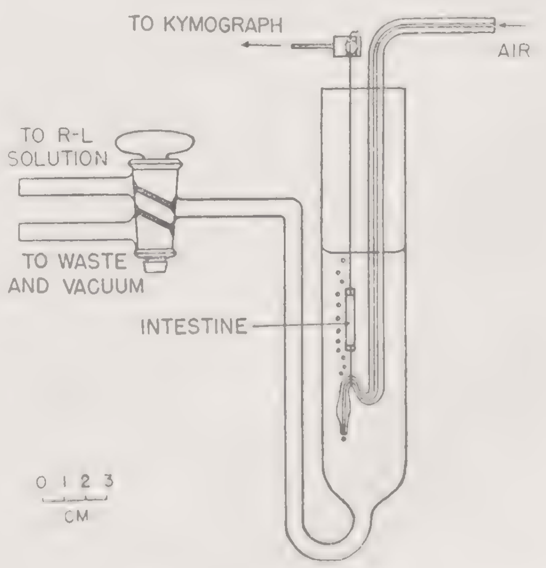

FIG. 32.—MUSCLE BATH

(g) *Histamine diphosphate std master soln.*—0.1 mg histamine diphosphate/ml in boiled H_2O. If stored in refrigerator when not in use, it will keep at least 3 months. Prep. dild stds of 0.01 mg/ml and 0.005 mg/ml as required. If bath smaller than 50 ml is used, prep. dilns of (g) with (f) to avoid diln of bath when stds are added.

(h) *Guinea pig intestine.*—Use guinea pigs weighing 300–400 g. Starve pig 24 hr, kill by blow on head, and remove intestine, severing at point proximal to ileocecal junction, retaining ca 12 cm of terminal ileum. Wash this section with soln (**f**) and use ca first 2 cm for first series of assays. Place remainder of intestine on cotton in petri dish, just covering with soln (**f**). Prop lid to admit air and store in refrigerator at ca 40–45°F. (Below 40°F intestine loses its activity.) Use addnl portions of intestine as required as long as material shows enough response to histamine stimulus (usually 8 days). These addnl portions of intestine are not as sensitive as ileum but give uniform response with barely noticeable pendulum movements after storage.

18.024 PREPARATION OF SAMPLE

Comminute sample (entire contents if canned) by passing 3 times thru food chopper, mixing after each grinding. Freeze ground sample for storage, if desired.

18.025 ASSAY

Weigh 10 g prepd sample into small mortar. Add enough H_2O to make smooth paste while grinding with pestle. Transfer paste with little addnl H_2O to 100 ml Kohlrausch flask and add 1 ml HCl (1+1). Add H_2O to total vol. of ca 70 ml, mix well, and heat flask in boiling H_2O bath ca 20 min. Remove from bath, cool, dil. to vol. with H_2O, mix, and filter on Whatman No. 12 folded paper (or equiv.). (Ext. filters slowly but only ca 5 ml need be collected for analysis.) Filtrate may be stored in refrigerator 10 days without diminished activity. Neutralize 1 ml filtrate with 2 ml 1% $NaHCO_3$ soln. Dil. to 10 ml with Ringer-Locke soln (without dextrose).

Attach intestine to muscle lever and let stand at least 0.5 hr in 50 ml Ringer-Locke soln, (**f**), in constant temp. bath at 37°. With fresh ileum, contractions and relaxations may be nonrhythmic for ca 2–3 hr with extreme and not always uniform responses to histamine stimuli. Detns may be performed during this period, but it is necessary to add small and increasing amounts of dild stds to stabilize intestine response, and to check responses several times until readings are reproducible.

Add known amount of dil. std soln, (**g**), to bath, record response, and remove writing lever from contact with drum. Drain inner bath, add fresh 37° soln (**f**) to wash chamber and intestine, remove, and refill with fresh soln. Let intestine rest 3 min. Estimate amount and diln of neutralized fish ext. that will give approx. equal response, and add to bath. Repeat recording of response, washing muscle chamber, and resting 3 min. During this interval, measure step heights with mm scale and calc. quantity std necessary to match assay step height. Add ext. and std alternately as above until exact match is obtained.

Chemical Method (16)—First Action

(Use H_2O redistd from glass for prepn of reagents and for detns. Do not clean glassware with soap; use fresh chromic acid cleaning soln, rinsing well with tap H_2O, then 3 times with distd H_2O, and 3 times with redistd H_2O. Alcohol may be used to soak or rinse glassware.)

18.026 REAGENTS

(**a**) *Benzene-n-butanol mixture.*—(3+2) v/v.

(**b**) *Cotton acid succinate.*—Dissolve 5 g anhyd. NaOAc, fused just before use, and 40 g succinic anhydride in 300 ml HOAc in 500 ml erlenmeyer. Immerse 10 g absorbent cotton, cut into strips, in soln; attach drying tube contg drying agent, and heat 48 hr at 100°. (Flask may be immersed to neck in active steam bath.) Filter; wash well with H_2O, HCl (1+9), H_2O, and finally with alcohol. Dry in vac. oven at 100°.

(**c**) *Diazonium reagent.*—Dissolve 0.1 g *p*-nitroaniline, recrystd from hot H_2O, and dil. to 100 ml with 0.1N HCl. Store in refrigerator. Dissolve 4 g $NaNO_2$ in H_2O and dil. to 100 ml. Store in refrigerator. Just before use place 10 ml of the *p*-nitroaniline soln in ice bath 5 min., add 1 ml of the $NaNO_2$ soln, mix, and let stand in bath at least 5 min. before use.

(**d**) *Coupling buffer.*—Dissolve 7.15 g Na metaborate ($NaBO_2$) and 5.7 g Na_2CO_3 in H_2O, and dil. to 100 ml. Store in polyethylene bottle.

(**e**) *Barbital buffer.*—Dissolve 10 g Na barbital in 1 L H_2O and adjust to pH 7.7 with HOAc (1+15) (ca 25–30 ml), using pH meter. Store in refrigerator to prevent mold growth. Dissolve any ppt by warming before use. (50–250 ml bottle of the buffer may be kept at room temp. and replenished from main supply when mold growth is apparent.)

(**f**) *Histamine std solns.*—Dry histamine.2HCl (USP Reference Standard or material checked against Standard as in **18.028**) 2 hr over H_2SO_4. Dissolve 0.1656 g dried histamine.2HCl in H_2O and dil. to 100 ml (1 ml = 1 mg histamine). Dil. 10 ml of this stock soln to 100 ml with H_2O (1 ml = 100 mmg histamine). Dil. 5 ml of this dil. std soln and 5 ml MeOH to 100 ml with H_2O (1

ml = 5 mmg histamine). Store in cold. Prep. fresh stds weekly.

(g) *4-Methyl-2-pentanone (methyl isobutyl ketone)*.—Commercial purified grade (Eastman No. 416 has been found satisfactory). To recover used ketone, wash once with satd $NaHCO_3$ soln and 3 times with H_2O, distill, retaining fraction boiling at 115–118°, and check absorbance at 475 mμ.

(h) *Benzaldehyde*.—Cl-free.

(i) *Dilute sulfuric acid*.—$0.40 \pm 0.02N$, accurately stdzd.

18.027 PREPARATION OF CAS COLUMN

Prep. column by firmly placing small plug of cotton acid succinate (CAS) (ca 50 mg) in column prepared by cutting off or blowing out bottom of 15 ml centrifuge tube. Wash plug with three 15 ml portions H_2O and two 3 ml portions alcohol. Let solvents drip thru CAS, syringing out column by blowing out last portion of each solvent, using 10 ml syringe with needle inserted thru rubber stopper. CAS plugs may be re-used for months by washing shortly after use with H_2O and alcohol as above, and protecting from dust with inverted beaker.

18.028 DETERMINATION

Transfer 10 g prepd sample, **18.024**, to semimicro container of high-speed blender, add ca 50 ml MeOH, and blend ca 2 min. Transfer to 100 ml g-s. vol. flask, rinsing lid and blender jar with MeOH and adding rinsings to flask. Heat in H_2O bath to 60° and let stand at this temp. 15 min. Cool to 25°, dil. to vol. with MeOH, and filter thru folded paper. Alcohol filtrate may be stored in refrigerator several weeks. (Light powdery ppt separating on storage may be ignored.)

Dil. 5 ml filtrate to 100 ml with H_2O (disregard turbidity). Pipet 5 ml aliquot into 16×150 mm g-s. test tube, and add 1 drop benzaldehyde (Cl-free) and 0.2 ml 20% NaOH. (pH after adding alkali should be ca 12.4–12.5.) Shake vigorously ca 25 times. Let stand 2 min. and add 5 ml of the benzene-butanol mixt. Shake vigorously ca 25 times and let stand 5 min. to sep. If emulsion forms, centrifuge.

Transfer upper layer with fine-tip tube equipped with rubber bulb to previously prepd CAS column, avoiding transfer of any aq. phase. Re-ext. aq. soln with 5 ml of the benzene-butanol mixt. as before, shaking, letting stand 5 min., and transferring upper layer to column. Rinse lip and sides of column with fine stream of alcohol from wash bottle, syringing out CAS. Wash column with 3 ml alcohol; syringe out; wash with two 3 ml portions H_2O, and syringe out. Discard solvents and washings.

Elute histamine from CAS into 25 ml g-s. erlenmeyer by washing down sides of tube with 2.0 ml $0.40 \pm 0.02N$ H_2SO_4 (vol. and concn of acid are critical) followed by 3 ml H_2O. Syringe out after dripping ceases.

Cool eluate in ice bath, weighting flask with lead ring or clamp to prevent tipping, and let stand 5–10 min. Add 0.5 ml cooled diazonium reagent and let stand 5 min. in ice bath. Add 0.50 ml coupling buffer (vol. is critical; Ostwald pipet is convenient) with continuous shaking or swirling to avoid localized alkalinity (pH after addn of coupling buffer, 5–6). Let stand 5 min. in ice bath. Sat. soln with ca 0.25 g powd. $Na_2B_4O_7.10H_2O$ added in one portion. Shake soln immediately and continuously ca 30 sec. to insure rapid and complete satn (final pH ca 8.6). Let stand in ice bath 15 min.

Pipet in 5.0 ml methyl isobutyl ketone and shake vigorously 25 times. Immediately transfer both layers to 16×150 mm test tube (do not rinse) and let stand 10 min. at room temp. to sep. and to warm up. Transfer upper layer with fine-tip dropper to second 16×150 mm g-s. test tube contg 5.0 ml of the barbital buffer. Avoid transferring aq. and solid phases (if present) (transfer need not be quant.). Shake vigorously ca 25 times (pH of barbital buffer after washing, ca 8.3–8.4). Let stand 10 min. to sep.

Transfer upper layer with fine-tip dropper to 1 cm cell and det. absorbance at 475 mμ against methyl isobutyl ketone. Repeat detn on samples yielding absorbance values higher than 25 mmg std by dilg 1 ml MeOH filtrate to 100 ml with H_2O. Alternatively, aq. diln may be dild 1+4 (or more) with H_2O.

Conduct std and blank thru detn as follows: Pipet 5 ml of the 5 mmg/ml histamine std soln into 16×150 mm g-s. test tube and pipet 5 ml 5% MeOH into similar tube for blank. Proceed as in detn, beginning, par. 2, line 3, " . . . add 1 drop benzaldehyde . . . "

Subtract blank absorbance from absorbance of std (A') and of sample (A) and calc. histamine in sample aliquot as follows:

$$\text{Mmg histamine} = A(\text{corr.}) \times 25/A'(\text{corr.})$$

Indole in Shrimp, Oysters, and Crabmeat (17) First Action

18.029 APPARATUS AND REAGENTS

(a) *Color reagent*.—Dissolve 0.4 g p-dimethylaminobenzaldehyde in 5 ml HOAc and mix with 92 ml H_3PO_4 and 3 ml HCl. As purity of p-dimethylaminobenzaldehyde exerts strong influence on intensity of reagent blank, purify yellow commercial reagent as follows:

Dissolve 100 g in 600 ml HCl (1+6). Add 300

ml H_2O and ppt aldehyde by slowly adding 10% NaOH soln with vigorous stirring. As soon as pptd aldehyde appears white, stop addn of the NaOH soln, filter, and discard ppt. Continue neutralization until practically all aldehyde is pptd, but do not carry to completion, because last 4–5 g may be colored. Filter, and wash ppt with H_2O until washings are no longer acid. Dry aldehyde, which should be practically white, in desiccator.

(b) *Acetic acid, purified.*—If this reagent gives pink color with color reagent, purify by distg 500 ml in all-glass still with 25 g $KMnO_4$ and 20 ml H_2SO_4.

(c) *Dilute hydrochloric acid.*—Dil. 5 ml HCl to 100 ml with H_2O.

(d) *Indole std soln.*—Weigh accurately 20 mg indole into 200 ml vol. flask and dil. to mark with alcohol. Keep refrigerated and discard after 2 weeks.

(e) *Distillation apparatus.*—Use sep. steam generator for each unit. Steam generator may be made from 1 L erlenmeyer and connected to all-glass steam distn app. with min. use of rubber tubing. Distn flask (capacity not <500 ml) is connected to straight bore condenser thru spray trap. 500 ml erlenmeyer is effective receiver. Foil-covered rubber stoppers may be used in absence of all-glass app. (Unprotected natural or synthetic rubber connections and stoppers cause variable distn blanks.)

Guard against traces of Cl in the H_2O, as they may partly or entirely inhibit development of indole color.

18.030 PREPARATION OF SAMPLE

Crabmeat, oysters, and shrimp.—For oyster meats weigh 50 g; for drained crabmeat or peeled raw or cooked shrimp, weigh 25 or 50 g (depending upon quantity of indole expected). Transfer weighed portion to high speed blender, add 80 ml H_2O (if oysters or crabmeat) or 80 ml alcohol (if shrimp), and mix several min. until homogeneous. Transfer mixt. quantitatively to distn flask, and rinse mixing chamber with min. quantity of same solvent used for prepg mixt.

18.031 DETERMINATION

Connect flask for steam distn and gently apply steam until distn is well started, using care not to pass in steam so vigorously as to cause excessive foaming. Apply enough heat to distn flask to maintain vol. of 80–90 ml. Collect 350 ml distillate in ca 45 min. (If alcohol was used in prepn of sample, collect 450 ml.) Wash condenser with small quantity of alcohol and allow to drain into receiving flask contg distillate.

Transfer distillate to 500 ml separator and add 5 ml of the dil. HCl and 5 ml satd Na_2SO_4 soln. Ext. successively with 25, 20, and 15 ml portions $CHCl_3$, shaking vigorously at least 1 min. each time. Combine the 25 and 20 ml exts in 500 ml separator and wash with 400 ml H_2O, 5 ml satd Na_2SO_4 soln, and 5 ml of the HCl. Save wash H_2O. Filter combined exts thru cotton plug into dry 125 ml separator. Wash the 15 ml portion, using same wash H_2O, and combine with other portions in same 125 ml separator.

Add 10 ml of the color reagent to combined exts, shake vigorously exactly 2 min., and let acid layer sep. as completely as possible. Transfer 9.0 ml acid layer to 50 ml vol. flask, dil. to mark with HOAc, mix well, transfer soln to suitable photometer cell, and measure color photometrically at 560 mμ. Color soln may be dild with HOAc contg 9.0 ml color reagent/50 ml of soln, provided blanks are detd at same dilns.

Prep. std curve as above by steam distg series of freshly prepd dilns of std indole soln. Det. distn blank similarly, omitting addn of indole.

Paralytic Shellfish Poison

Biological Method (18)—Official

18.032 MATERIALS

(a) *Paralytic shellfish poison std soln.*—100 mmg/ml. Available from Shellfish Sanitation Section, Public Health Service, Washington 25, D.C., as acidified 20% alc. soln. Std is stable indefinitely in cool place.

(b) *Paralytic shellfish poison reference soln.*—1 mmg/ml. Dil. 1 ml std soln to 100 ml with H_2O. Soln is stable several weeks at 3–4°.

(c) *Mice.*—Healthy mice, 19–21 g, from stock colony used for routine assays. If <19 g or >21 g, apply correction factor to obtain true death time (see Table). Do not use mice weighing >23 g and do not reuse mice.

18.033 STANDARDIZATION OF BIOASSAY

Dil. 10 ml aliquots of 1 mmg/ml reference soln with 10, 15, 20, 25, and 30 ml H_2O, resp., until intraperitoneal injection of 1 ml doses into few test mice causes median death time of 5–7 min. pH of dilns should be 2–4 and must not be >4.5. Test addnl dilns in 1 ml increments of H_2O, e.g., if 10 ml dild with 25 ml H_2O kills mice in 5–7 min., test solns dild 10+24 and 10+26.

Inject group of 10 mice with each of 2 or preferably 3 dilns that fall within median death time of 5–7 min. Give 1 ml dose to each mouse by intraperitoneal injection and det. death time as time elapsed from completion of injection to last gasping breath of mouse.

Repeat assay 1 or 2 days later, using dilns prepd above which differed by 1 ml increments

of H_2O. Then repeat entire test, starting with testing of dilns prepd from newly prepd reference soln.

Calc. median death time for each group of 10 mice used on each diln. If all groups of 10 mice injected with any 1 diln gave median death time <5 or >7 min., disregard results from this diln in subsequent calculations. On other hand, if any of the groups of 10 mice injected with 1 diln gave median death time falling between 5 and 7 min., include all groups of 10 mice used on that diln, even though some of the median death times may be <5 or >7 min. From median death time for each group of 10 mice in each of selected dilns, det. number of mouse units/ml from Sommer's Table. Divide calcd mmg poison/1 ml by mouse units/1 ml to obtain conversion factor (CF value) expressing mmg poison equiv. to 1 mouse unit. Calc. av. of individual CF values, and use this av. value as reference point to check routine assays. Individual CF values may vary significantly within laboratory if techniques and mice are not rigidly controlled. This situation will require continued use of reference std or secondary std, depending on vol. of assay work performed.

18.034 USE OF STANDARD WITH ROUTINE ASSAYS OF SHELLFISH

Check CF value periodically as follows: If shellfish products are assayed less than once a week, det. CF value on each day assays are performed by injecting 5 mice with appropriate diln of reference std. If assays are made on several days during week, only 1 check need be made each week on diln of std such that median death time falls within 5–7 min. CF value thus detd should check with av. CF value within ±20%. If it does not check within this range, complete group of 10 mice by adding 5 mice to the 5 mice already injected, and inject second group of 10 mice with same diln of std. Average CF value detd for second group with that of first group. Take resulting value as new CF value. Variation of >20% represents significant change in response of mice to poison, or in technique of assay. Changes of this type require change in CF value.

Repeated checks of CF value ordinarily produce consistent results within ±20%. If wider variations are found frequently, possibility of uncontrolled or unrecognized variables in method should be investigated before proceeding with routine assays.

18.035 PREPARATION OF SAMPLE

(**a**) *Clams, oysters, and mussels.*—Thoroly clean outside of shellfish with fresh H_2O. Open by cutting adductor muscles. Rinse inside with fresh H_2O to remove sand or other foreign material. Remove meat from shell by sepg adductor muscles and tissue connecting at hinge. Do not use heat or anesthetics before opening shell, and do not cut or damage body of mollusk at this stage. Collect ca 100–150 g meats in glazed dish. As soon as possible, transfer meats to No. 10 sieve without layering, and let drain 5 min. Pick out pieces of shell and discard drainings. Grind in household-type grinder with $\frac{1}{8}$–$\frac{1}{4}$″ holes, or macerate in blender until homogeneous.

(**b**) *Scallops.*—Sep. edible portion (adductor muscle) and apply test to this portion alone. Drain and grind as in (**a**).

(**c**) *Canned shellfish.*—Place entire contents of can (meat and liquid) in blender and macerate until homogeneous. For large cans, drain meat in large büchner or sieve and collect all liquid. Det. wt of meat and vol. of liquid. Recombine portion of each in proportionate quantities. Macerate recombined portions in blender until homogeneous.

18.036 EXTRACTION

Weigh 100 g well-mixed material into tared beaker. Add 100 ml 0.1*N* HCl, stir thoroly, and check pH. (pH should be <4.0, preferably ca 3.0. If necessary, adjust pH as indicated below.) Heat mixt., boil gently 5 min., and let cool to room temp. Adjust cooled mixt. to pH 2.0–4.0 (never >4.5) as detd by *BDH Universal Indicator*, *phenol blue*, *Congo red paper*, or pH meter. To lower pH add 5*N* HCl dropwise with stirring; to raise pH add 0.1*N* NaOH dropwise with constant stirring to prevent local alkalinization and consequent destruction of poison. Transfer mixt. to graduated cylinder and dil. to 200 ml.

Return mixt. to beaker, stir to homogeneity, and let settle until portion of supernatant is translucent and can be decanted free of solid particles large enough to block 26-gauge hypodermic needle. If necessary, centrifuge mixt. or supernatant 5 min. at 3000 rpm or filter thru paper. Only enough liquid to perform bioassay is necessary.

18.037 MOUSE TEST

Inoculate each test mouse intraperitoneally with 1 ml of the acid ext. Note time of inoculation and observe mice carefully for time of death as indicated by last gasping breath. Record death time from stopwatch or clock with sweep second hand. One mouse may be used for initial detn, but 2 or 3 are preferred. If death time or median death time of several mice is <5 min., make diln to obtain death times of 5–7 min. If death time of 1 or 2 mice injected with undild sample is >7 min., total of at least 3 mice must be inoculated to establish toxicity of sample. If large dilns are necessary, adjust pH of diln by dropwise addn of

dil. HCl (0.1 or 0.01*N*) to pH 2.0–4.0 (never >4.5). Inoculate 3 mice with diln that gives death times of 5–7 min.

18.038 CALCULATION OF TOXICITY

Det. median death times of mice, including survivors, and from Sommer's table det. corresponding number of mouse units. If test animals weigh <19 g or >21 g, make correction for each mouse by multiplying mouse units corresponding to death time for that mouse by wt correction factor for that mouse from Sommer's Table; then det. median mouse unit for group. (Consider death time of survivors as >60 min. or equiv. to <0.875 mouse unit in calcg median.) Convert mouse units to mmg poison/ml by multiplying by CF value.

Mmg poison/100 g meat = (mmg/ml × diln factor) × 200.

Consider any value >80 mmg/100 g as hazardous and unsafe for human consumption.

18.039 **Sommer's Table**

Death time: mouse unit relations for paralytic shellfish poison (acid)

DEATH TIME[a]	MOUSE UNITS
1:00	100
10	66.2
15	38.3
20	26.4
25	20.7
30	16.5
35	13.9
40	11.9
45	10.4
50	9.33
55	8.42
2:00	7.67
05	7.04
10	6.52
15	6.06
20	5.66
25	5.32
30	5.00
35	4.73
40	4.48
45	4.26
50	4.06
55	3.88
3:00	3.70
05	3.57
10	3.43
15	3.31
20	3.19
25	3.08
30	2.98
35	2.88
40	2.79
45	2.71
50	2.63
55	2.56
4:00	2.50
05	2.44
10	2.38
15	2.32
20	2.26
25	2.21
30	2.16
35	2.12
40	2.08
45	2.04
50	2.00
55	1.96
5:00	1.92
05	1.89
10	1.86
15	1.83
20	1.80
30	1.74
40	1.69
45	1.67
50	1.64
6:00	1.60
15	1.54
30	1.48
45	1.43
7:00	1.39
15	1.35
30	1.31
45	1.28
8:00	1.25
15	1.22
30	1.20
45	1.18

Sommer's Table Continued

DEATH TIME[a]	MOUSE UNITS
9:00	1.16
30	1.13
10:00	1.11
30	1.09
11:00	1.075
30	1.06
12:00	1.05
13	1.03
14	1.015
15	1.000
16	0.99
17	0.98
18	0.972
19	0.965
20	0.96
21	0.954
22	0.948
23	0.942
24	0.937
25	0.934
30	0.917
40	0.898
60	0.875

[a] Minutes: Seconds.

Correction table for weight of mice

WT OF MICE, G	MOUSE UNITS
10	0.50
10.5	0.53
11	0.56
11.5	0.59
12	0.62
12.5	0.65
13	0.675
13.5	0.70
14	0.73
14.5	0.76
15	0.785
15.5	0.81
16	0.84
16.5	0.86
17	0.88
17.5	0.905
18	0.93
18.5	0.95
19	0.97
19.5	0.985
20	1.000
20.5	1.015
21	1.03
21.5	1.04
22	1.05
22.5	1.06
23	1.07

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19. Flavoring Extracts

VANILLA EXTRACT AND ITS SUBSTITUTES

19.001 Specific Gravity—Official

Det. sp. gr. at 20/20° with pycnometer as in **9.011**.

19.002 Alcohol—Official

Proceed as in **9.013** or **9.021**, but measure sample at 15.56° in pycnometer, Fig. 18, calibrated at that temp.

19.003 Glycerol—First Action

Proceed as in **11.012** or **11.013**, selecting method according to quantity of sugar present. Use quantity of sample contg 0.1–0.4 g glycerol.

Propylene Glycol (*1*)—First Action

19.004 APPARATUS

All-glass distn app. with ₮ 24/40 joints: 250 ml erlenmeyer, 20 ml Barrett H_2O trap with ₮ stopcock, and West condenser with drip tip.

19.005 REAGENTS

(**a**) *Heptane.*—Eastman practical grade, b. p. 96–100°, or equiv.

(**b**) *Potassium arsenite std soln.*—0.02*N*. Dissolve 4.9455 g reagent As_2O_3, pulverized and dried to constant wt at 100°, in 75 ml 1*N* KOH. Add 40 g $KHCO_3$, dissolved in ca 200 ml H_2O, and dil. with H_2O to 1 L at 25°. Dil. 200 ml of this soln to 1 L with H_2O.

(**c**) *Potassium periodate std soln.*—0.02*M*. Dissolve 4.6 g KIO_4 in ca 500 ml hot H_2O. Dil. to ca 900 ml with H_2O, cool to room temp., and dil. to 1 L. Stdze frequently since this soln decomposes on standing.

(**d**) *Bromocresol purple indicator soln.*—Dissolve 0.1 g indicator in 100 ml alcohol and filter if necessary.

(**e**) *Propylene glycol.*—Reagent grade or commercial product which meets following test: Dil. 0.5 ml to 25 ml with H_2O, add 25 ml of the 0.02*M* KIO_4 soln, and let stand 10 min. Titr. with 0.02*N* NaOH, using 3 drops of the bromocresol purple. Vol. NaOH soln consumed minus end point correction obtained by titrg 50 ml H_2O should not be >0.1 ml.

19.006 ISOLATION OF PROPYLENE GLYCOL

Place sample contg ca 1 g propylene glycol in 250 ml ₮ erlenmeyer; add enough H_2O, if necessary, to make total vol. 10 ml. Add 60 ml heptane, few glass beads, and/or SiC grains. Connect flask to receiver attached to condenser. Fill receiver with heptane, heat flask with variable heat hot plate, and reflux at such rate that rapid stream of distillate flows from tip. Reflux ca 8 hr and cool.

Open stopcock of receiver and transfer aq. layer to 250 ml (or other convenient size) vol. flask. Wash condenser, receiver, and solvent layer by pouring six 10 ml portions H_2O down condenser, collecting each portion in receiver, and draining it into vol. flask. Finally wash with enough H_2O (ca 25 ml) to completely fill receiver, causing solvent layer to return to distn flask. Dil. to vol. and mix well.

19.007 DETERMINATION

(**a**) *Glycerol absent.*—Place aliquot of the aq. soln contg not >45 mg propylene glycol in g-s. flask, add 35 ml of the 0.02*M* KIO_4 soln, dil. to ca 100 ml with H_2O, and let stand 1 hr. Add ca 1.0 g $NaHCO_3$, 0.5 g KI, and 2.5 ml starch indicator, **2.093**(**d**). Titr. with the 0.02*N* $KAsO_2$ soln to the disappearance of blue color. Stdze 25 ml of the 0.02*M* KIO_4 soln by the same titrn procedure and calc. amount of KIO_4 reduced by sample. 1 ml 0.02*N* $KAsO_2$ = 0.76 mg propylene glycol.

(**b**) *Glycerol present.*—Proceed as in (a). If I is not liberated on addn of $NaHCO_3$ and KI, insufficient KIO_4 was present. Repeat detn, using smaller aliquot or increasing vol. of KIO_4 soln.

To det. glycerol in the aq. soln, place same vol. aliquot used above in g-s. flask, add 1 drop bromocresol purple, and add 0.02*N* NaOH soln to light purple color. Add same vol. KIO_4 soln used above, dil. to ca 100 ml, and let stand 1 hr. Add 10 drops propylene glycol (ca 0.5 ml), mix well, wash down sides of flask with H_2O, and let stand 10 min. Add 3 drops of the indicator and titr. with 0.02*N* NaOH to light purple end point. Titr. rapidly but do not shake flask violently in order to avoid excessive absorption of interfering CO_2 from air. Det. blank for this detn by repeating above procedure, using H_2O in place of sample and omitting 1 hr standing. Subtract blank from titrn obtained for sample aliquot. 1 ml 0.02*N* $KAsO_2$ = 0.46 mg glycerol; 1 ml 0.02*N* NaOH = 1.84 mg glycerol.

$$\text{Mg propylene glycol in aliquot} = [\text{ml } 0.02N\ KAsO_2 - (4 \times \text{ml } 0.02N\ NaOH)] \times 0.76$$

Vanillin—Official

Photometric Method (2)

(Applicable to vanilla, vanilla contg added vanillin and/or coumarin, and imitation vanilla)

19.008 REAGENTS

(a) *Folin-Denis reagent.*—*See* **9.051**(a).

(b) *Sodium carbonate soln.*—Dissolve 40 g Na_2CO_3 in 160 ml H_2O.

(c) *Lead acetate soln.*—Dissolve 50 g each of neutral and basic $Pb(OAc)_2$ in hot H_2O, dil. to 1 L, cool, and filter.

(d) *Vanillin std soln.*—Dissolve 0.1000 g vanillin in 3 ml alcohol in 100 ml vol. flask, and dil. to mark with H_2O. 1 ml = 1 mg vanillin.

19.009 PREPARATION OF STANDARD CURVE

Place 0.0, 2.5, 5.0, 7.5, 12.5, and 20.0 ml std vanillin soln in 500 ml vol. flasks. (Use 250 ml flasks if neutral wedge photometer is to be used for color reading.) To each flask add H_2O to total vol. of ca 80 ml and then add, from graduated pipet, 2.0 ml of the $Pb(OAc)_2$ soln. Dil. to mark with H_2O, mix, and filter thru dry 18.5 cm fluted paper (Whatman No. 12 or equiv.), discarding cloudy filtrate. Pipet 10 ml clear filtrate into 100 ml vol. flask, add 5 ml of the Folin-Denis reagent, mix, and let stand exactly 5 min. after mixing. Then add 10 ml of the Na_2CO_3 soln, mix, and let stand exactly 10 min. Dil. to mark with H_2O, mix, and filter thru dry fluted paper (Whatman No. 12 or equiv.), discarding cloudy filtrate. Immediately read absorbance or transmittance at 610 mμ and plot reading against concn. Curve may not obey Beer's law. With neutral wedge photometer, use 1″ cell and filter 61 or 65.

19.010 DETERMINATION

Pipet 2 ml sample into 500 ml vol. flask (250 ml if neutral wedge photometer is to be used) and proceed as in **19.009** concurrently with std series. If vanillin concn is >1.0 g/100 ml sample, dil. 50 ml sample to 100 ml with H_2O and use dild sample.

Coumarin—Official

Photometric Method (3)

(Applicable to vanilla, vanilla contg added vanillin and/or coumarin, and imitation vanilla)

19.011 REAGENTS

(a) *Sodium carbonate soln.*—Dissolve 5 g Na_2CO_3 in H_2O and dil. to 500 ml with H_2O.

(b) *Diazonium reagent.*—Prep. following solns: (1) Dissolve 0.7 g *p*-nitraniline in 9 ml HCl and dil. to 100 ml with H_2O. (2) Dissolve 5 g $NaNO_2$ in H_2O and dil. to 100 ml with H_2O. Chill both solns and 100 ml vol. flask to ca 3°. Pipet 5 ml of each soln into flask, mix, and let stand 5 min. at ca 3°. Add 10 ml more of soln (2), let stand 5 min. at ca 3°, and dil. to mark with ice-cold H_2O. Let stand 15 min. and discard after 24 hr.

(c) *Coumarin std soln.*—Dissolve 0.2000 g coumarin in 3 ml alcohol in 100 ml vol. flask and dil. to 100 ml with H_2O. 1 ml = 2 mg coumarin.

19.012 PREPARATION OF STANDARD CURVE

Pipet 0.0, 1.0, 2.0, 3.0, 4.0, and 5.0 ml std coumarin soln into 200 ml vol. flasks. (Use 100 ml flasks if neutral wedge photometer is to be used for color reading.) To each flask add H_2O to total vol. of ca 80 ml and then add 5 ml $Pb(OAc)_2$ soln, **19.008**(c). Dil. to mark with H_2O, mix, and filter thru 18.5 cm fluted paper (Whatman No. 12 or equiv.), discarding cloudy filtrate. Dissolve 0.2 g anhyd. Na oxalate in the clear filtrate by swirling, let stand at least 5 min., and filter thru 18.5 cm fluted paper, discarding cloudy filtrate.

Pipet 5 ml clear filtrate into 100 ml vol. flask and add 15 ml H_2O and 10 ml of the Na_2CO_3 soln. Heat in boiling H_2O bath 5 min. and let cool gradually. When room temp. is reached, add 10 ml of the diazonium reagent, dil. to mark with H_2O, and mix. Let stand 1.5 hr, filter thru 18.5 cm fluted paper, read absorbance or transmittance at 490 mμ, and plot reading against concn. With neutral wedge photometer, use 0.5″ cell and filter 49.

19.013 DETERMINATION

Pipet 5 ml sample into 200 ml vol. flask (100 ml if neutral wedge photometer is to be used) and proceed as in **19.012** concurrently with std series.

Vanillin, Ethyl Vanillin, and Coumarin (4)—Official

Chromatographic Separation Method

19.014 APPARATUS

(a) *Spectrophotometer.*—Capable of detg absorbance at 270 and 325 mμ. Adjust to high sensitivity to utilize slit width <10 mμ.

(b) *Silica cells.*—1 cm. Match cells at 270 and 325 mμ, using *iso*-octane-$CHCl_3$ solvent, **19.015**(c). (This solvent dets differences that other media do not.) Cells must be free of other solvents before adding the *iso*-octane-$CHCl_3$ solvent, std, and sample solns. Drain each cell well between readings by inverting on towel. Fill cells for reading so that meniscus is >3 mm above light path.

(c) *Chromatographic tube.*—Melt glass tube 11–12.5 mm i. d. ca 18″ from one end, draw out short distance, cool, and break at constriction. Partially close constricted end in flame, and dry.

19.015 REAGENTS

(**a**) *Silicic acid.*—Reagent grade "100-mesh" powder, suitable for chromatography (Mallinckrodt Chemical Co. No. 2847 or equiv.). Det. SiO_2 content as follows: Weigh accurately ca 1 g silicic acid into weighed Pt crucible. Ignite in muffle 15 min. at 615°, cool in desiccator, and reweigh. Calc. % SiO_2 (z) in the silicic acid. Calc. quantity of silicic acid (x) required for 5.8 g column from equation: $x = 3.384 \times 100/z$. Quantity of H_2O (y) required for column is $5.80 - x$.

(**b**) *iso-Octane.*—Practical grade 2,2,4-trimethylpentane, 99.5+%, b. p. 98–100°.

(**c**) *iso-Octane-chloroform solvent mixture.*—Add 40 ml $CHCl_3$ to 1 L *iso*-octane and mix. Store in air-tight bottle. (Do not use rubber stopper.) Soln contains ca 3.85% $CHCl_3$.

(**d**) *Coumarin std soln.*—Accurately weigh 100 mg coumarin into 100 ml vol. flask, dissolve in 50 ml $CHCl_3$, and dil. to mark with *iso*-octane. (1 ml = 1 mg coumarin.)

(**e**) *Ethyl vanilin std soln.*—Prep. as in (**d**) using Et vanillin.

(**f**) *Vanillin std stoln.*—Prep. as in (**d**) using vanillin.

19.016 DETERMINATION OF ABSORPTIVITIES

Pipet 1 ml vanillin std soln into 100 ml vol. flask, add 3.4 ml $CHCl_3$, dil. to vol. with *iso*-octane, and mix. Det. absorbance, A, at 270 and 325 mμ against solvent, **19.015(c)**, as reference in the 1 cm silica cells. Calc. absorptivity, a (g/L; 1 cm) for vanillin at 270 and at 325 mμ from equation: $a = 100A$.

Det. absorptivities for Et vanillin and vanillin similarly.

19.017 PREPARATION OF CHROMATOGRAPHIC COLUMN

Pack small cotton wad in bottom of *dry* chromatographic tube. To x g silicic acid in mortar add from buret y ml H_2O, mix thoroly and quickly to uniform powdery consistency with pestle, and immediately add 25 ml solvent, **19.015(c)**. Mix and pour slurry rapidly thru funnel into tube. Rinse mortar and funnel with small vol. solvent. Remove any air bubbles formed by stirring with long thin glass rod. Pack column with ca 2 lbs/sq. in. air pressure until bottom of meniscus of free solvent just touches top surface of silicic acid but outer part of meniscus is still clearly visible. *Immediately release pressure.* (IMPORTANT: If column channels or cracks, discard. During packing and thereafter, keep column vertical. Tipping ruins column for further use altho it may appear normal.) Carefully add 15 ml of the solvent down side of tube with aid of glass rod so column is not disturbed. Drive solvent thru column. Washed column is now ready for calibration.

19.018 CALIBRATION OF COLUMN

Pipet and combine 1 ml of each std soln, **19.015(d)**, (**e**), and (**f**), in 25 ml vol. flask. Dil. to mark with *iso*-octane and mix. Pipet 2 ml of the soln down one side of chromatographic tube onto top of column. Drive soln into column with ca 2 lbs air pressure and collect eluate in 10 ml graduated cylinder. Release pressure when bottom of meniscus touches top of column and outer part of meniscus is still clearly visible. Pipet 1 ml solvent, **19.015(c)**, down same side of tube onto column and drive into column. Repeat with 2 addnl 1 ml portions solvent. Fill tube to within 1″ of top with solvent. Drive solvent thru column at rate of 5 ml /2–2.5 min., collecting 5 ml eluate fractions, alternating two 10 ml graduated cylinders during collection. Pour fractions into sep. test tubes in rack, numbering fractions consecutively. Drain cylinder before reusing by inverting on towel. Collect 10 fractions and det. absorbances at 270 and 325 mμ against solvent **19.015(c)**, as reference in 1 cm silica cells. Drain cells by inverting on towel before refilling; rinsing is not necessary. Permit column to elute by gravity while reading first 10 fractions, changing cylinders for each 5 ml portion.

Coumarin elutes first, Et vanillin second, and vanillin third. In ideal column coumarin begins to elute in fraction 6–7, reaches max. in 8–9, and fades considerably in 10. Earlier elution does not sep. compounds entirely; later elution takes more fractions and time but does give good recoveries. Somewhat slower elution does not matter. Et vanillin elutes in ca fraction 11–18, and vanillin in ca 19–30.

If column is satisfactory, collect 25 addnl fractions (35 in all) or until vanillin is completely eluted. Det. absorbances of each fraction at 270 and 325 mμ as above.

If coumarin begins to elute at fraction 5 or earlier, discard, prep. another column with less H_2O in the silicic acid, and recalibrate. If coumarin does not elute by fraction 9–10, prep. new column with more H_2O in the silicic acid.

Use calibrated column for detn, **19.021**.

19.019 PREPARATION OF SAMPLE SOLUTION

If concn of none of detd compounds is >0.4 g/100 ml, pipet 25 ml sample into 250 ml centrifuge bottle. If concn of any detd compound is >0.4 g/100 ml, dil. 25 ml sample with H_2O to vol. specified in table, **19.020**, and use 25 ml aliquot. Add 75 ml H_2O, 20 ml H_2SO_4 (1+4), and 50 ml $CHCl_3$. Stopper with rubber stopper and shake well 3 min. Centrifuge 5 min. at 1500 rpm. If emulsion persists, break with thin glass rod and

recentrifuge. Pour contents slowly thru large-bore, short-stem funnel into 250 ml separator. Break emulsion with glass rod and drain $CHCl_3$ into 100 ml vol. flask. Pour aq. phase thru same funnel back into bottle.

Rinse separator with 15 ml $CHCl_3$, add to bottle thru funnel, and repeat extn by mixing phases thoroly with rocking motion. Do not shake vigorously as in first extn. Centrifuge, sep., and drain $CHCl_3$ into 100 ml vol. flask. Repeat extn with 15 ml portions $CHCl_3$ until flask is filled to mark.

19.020 *Dilns and diln factors for flavorings*

CONCN OF MOST ABUNDANT CONSTITUENT, G/100 ML	DIL. TO: (ML)	DILN FACTOR F
<0.4	none	12.5
0.4–0.8	50	25.0
0.8–1.6	100	50.0
1.6–3.2	200	100
>3.2	200	200

19.021 IDENTIFICATION

Pipet 2 ml of the sample soln (use 1 ml if concn of most abundant compound is >3.2 g/100 ml) onto prepd column, letting it flow down one side of tube without disturbing column. Drive soln into column with ca 2 lbs air pressure and collect eluate in 10 ml graduated cylinder. Pipet 1 ml solvent, **19.015(c)**, down same side of tube and drive into column. Repeat with 2 addnl 1 ml portions of the solvent. Fill tube with solvent and elute compounds by same procedure and conditions as for calibration. Collect 3 more fractions than indicated necessary by calibration. Det. absorbances of all fractions at 270 and 325 mμ as in calibration.

Positions of absorbing fractions compared to those obtained during column calibration reveal compounds present in sample. Coumarin is also identified by absorbing at 325 mμ slightly $>\frac{1}{3}$ its absorbance at 270 mμ. Vanillin and Et vanillin absorb very little at 325 mμ. If desired, confirm by obtaining ultraviolet spectrum of one high absorbing fraction of each compound. Compare with spectra prepd on same instrument. Spectra of compounds exhibit approx. max. and min. given below. Approx. ratio of absorbance at given wavelength to that at highest max. for respective compound is given in parentheses after that wavelength. Ratio of 1.00 indicates highest max.

19.022 DETERMINATION

Add absorbances at 270 mμ of all fractions contg coumarin and calc. coumarin concn in original sample from equation:

$$c = F \times \Sigma A \times a,$$

where ΣA is sum of absorbances at 270 mμ of fractions contg coumarin, a is absorptivity of coumarin at 270 mμ, c is concn coumarin in g/100 ml original sample, and F is diln factor, given in **19.020**.

Calc. concns of Et vanillin and vanillin similarly.

If negative absorbances are obtained on fractions not contg the compounds, correct absorbances of fractions contg compounds by adding to each absorbance the av. of the negative readings.

NOTE: Same batch of *iso*-octane must be used to prep. solvent, **19.015(c)**, and all dilns for set of detns.

Lead Number (Wichmann) (*5*)—Official

19.023 PREPARATION OF SAMPLE SOLUTION

Place 175 ml boiled H_2O in round-bottom 1 L flask. Add, by pipet, 25 ml clear $Pb(OAc)_2$ soln (8 g/100 ml) and 50 ml sample. Place flask in hole in asbestos board large enough to prevent heating upper portion of flask. (When contents of flask are reduced to 50 ml of liquid, level of liquid should be even with top of board, or slightly above it.)

Connect flask to condenser, and with moderate flame distill 200 ml into vol. flask, reserving distillate for detn of alcohol. Transfer residue to 100 ml vol. flask with CO_2-free H_2O and bent glass rod provided with rubber tip. When cool, dil. to 100 ml with CO_2-free H_2O, mix, and filter thru dry filter (Soln *A*).

Conduct blank detn, using 5 drops of HOAc in place of sample and distg 150 ml instead of 200 ml. Det. Pb as in **19.024(a)** or **(b)**; calc. Pb number and report as "Lead Number—Wichmann."

19.024 DETERMINATION OF LEAD

(a) *As sulfate.*—Pipet 10 ml Soln *A*, **19.023**, into 250 ml beaker and add 25 ml H_2O, 2 ml H_2SO_4 (1+1), and 100 ml alcohol; stir and let settle overnight. Filter on gooch, wash with alcohol, ignite at low redness, cool in desiccator, and weigh. (Wt $PbSO_4$ obtained from blank − wt obtained from sample) $\times 13.66 =$ Pb number.

(b) *As chromate.*—Pipet 10 ml Soln *A*, **19.023**, into 400 ml beaker and add 2 ml HOAc, 25 ml

COMPOUND	MAXIMA, Mμ			MINIMA, Mμ		
Coumarin	271 (1.00)	282 (0.82)	313 (0.46)	243 (0.30)	278.5 (0.81)	291.5 (0.31)
Et vanillin	270 (1.00)	297 (0.59)		242.5 (0.24)	286 (0.46)	—
Vanillin	269.5 (1.00)	296 (0.60)	—	242.5 (0.24)	286 (0.48)	—

H_2O, and 25 ml ca 0.1*N* $K_2Cr_2O_7$. Heat beaker and contents immediately with moderate flame until ppt changes in color from yellow to orange. Filter on gooch, wash thoroly with hot H_2O, and then with few ml each of alcohol and ether. Dry at 100°, cool in desiccator, and weigh. (Wt $PbCrO_4$ obtained from blank−wt obtained from sample)×12.82=Pb number.

19.025 Total Solids (*6*)**—Official**

Proceed as in **29.007** or **29.008,** using 10 ml sample.

19.026 Ash—Official

Evap. 10 ml ext. and proceed as in **29.012** or as in **29.013.**

19.027 Sucrose (*7*)**—Official—***See* **29.025, 29.026, or 29.032**

Vanilla Resins

19.028 *Quantitative Method* (*8*)*— First Action*

Pipet 50 ml sample into 150 or 250 ml beaker and dil. to 100 ml with H_2O. Boil rapidly on hot plate or over flame to vol. of ca 50 ml. Cool, and add NH_4OH (1+3) dropwise until slightly alk. Add 3 drops excess and stir vigorously 2 min. to insure soln of resins. Add HCl (1+1) dropwise, with stirring, until acid to indicator paper and then 2 ml excess. Stir, and let stand at room temp. at least 1 hr but not >24 hr. Add 0.5 g filter aid (Celite, Hyflo-Supercel) and filter with suction thru long stem, medium porosity fritted glass büchner funnel (30 ml capacity) contg pad prepd by pouring aq. suspension of 1 g filter aid thru funnel and washing with H_2O. If filtration slows, scratch surface of pad gently to break resin film. Transfer resins quantitatively to funnel with aid of policeman, using six 20 ml portions 0.05*N* HCl to wash beaker and funnel. Let each portion wash soln drain before adding next portion. Dry material as much as possible by suction, transfer funnel to dry suction flask, and dissolve resins from filter with boiling alcohol added in small portions, using some of alcohol to rinse beaker. Suck each portion thru funnel before adding next portion. Mix filter aid in funnel with the hot alcohol, using small glass rod. Repeat extns until alcohol soln is colorless. Rinse tip of funnel stem with hot alcohol and transfer soln quantitatively to weighed beaker or Pt dish. Evap. to dryness on steam bath and dry 1 hr at 100°. Cool in desiccator and weigh. Report results to 2 decimal places only. Reserve resins for qual. tests.

19.029 *Qualitative Tests* (*9*)*—Procedure*

Place portion of dried residue in few ml 5% KOH soln. (Vanilla resins dissolve, giving deep red soln.) Acidify, and ppt is obtained. Dissolve portion of dried residue in alcohol. To portion of soln add few drops 10% $FeCl_3$ soln; to another portion add HCl. Neither produces any marked change in color if residue consists of vanilla resins. Most other resins in alc. soln give color reactions with $FeCl_3$ or HCl.

To portion of filtrate obtained in **19.028** add few drops basic $Pb(OAc)_2$ soln, **29.021(a)**. Owing to excessive quantity of org. acids, gums, and other extractive matter, ppt is so bulky as almost to solidify. Filtrate from this ppt should be almost colorless. Test another portion of filtrate from the resin for tannin with *gelatin soln.* Tannin is present in varying small quantities, but should not be present in excessive quantities.

19.030 Methanol—Official

Proceed as in **9.043** or **9.044,** using distillate from alcohol detn, **19.002.**

19.031 Color Insoluble in Amyl Alcohol (*10*)**—First Action**

Evap. 25 ml sample just to dryness on steam bath. Dissolve residue in H_2O and alcohol, and dil. to vol. of 50 ml, using total vol. of 26.3 ml alcohol. Place 25 ml of this soln in separator and add 25 ml freshly shaken Marsh reagent, **9.047,** shaking lightly so as not to form emulsion. Let layers sep. completely, drain lower or aq. layer (which contains any caramel present) into 25 ml cylinder, and dil. to vol. with alcohol (50% by vol.). Compare this soln in colorimeter with untreated 25 ml. Calc. from this reading % color insol. in amyl alcohol.

Foreign Plant Material

Paper Chromatographic Method (*11*)*— First Action*

19.032 APPARATUS

Use ascending technic with small (8×8″) papers and solvents **19.033(a)** and **(b)**. Use descending technic with large ($22\frac{1}{4}\times18\frac{1}{8}$″) papers and solvents **19.033(c)**, **(d)**, and **(e)**.

(**a**) *For small sheets.*—See **24.097(a)**.

(**b**) *For large sheets.*—Use box suitable for this size paper chromatography. A satisfactory box is approx. 29.5×21.5×23.75″ covered with Formica or other material resistant to org. solvents, acid, alkali, etc. Lid of box is hinged and plastic gasket is used to obtain tight seal to prevent escape of vapors. Front of box contains window 11×17″ to observe progress of solvent front. Semicircular glass troughs ca 26.5×2″ are supported by stainless steel clamps on metal strips ca 2″ wide placed 2.5″ from top and extending from front to back along sides. Clamps also support glass rods on both sides of trough and parallel to it. (Unit

available from Chromatography Co., 811 Campus Way, Davis, Calif.)

19.033 SOLVENT SYSTEMS

(Listed in approx. order of usefulness)

(**a**) Dissolve 20 g KOH and 50 g KBr in H_2O, add 200 ml alcohol, and dil. to 1 L with H_2O.

(**b**) Dissolve 20 g $KHCO_3$ and 50 g KBr in H_2O, add 200 ml alcohol, and dil. to 1 L with H_2O.

(**c**) Shake 20 parts isobutanol, 0.8 part HOAc, and 15 parts H_2O in separator, let sep., and drain and discard lower layer. Use upper layer.

(**d**) Mix 8 parts isopropanol, 5 parts NH_4OH, and 15 parts H_2O.

(**e**) Mix 30 parts HOAc, 3 parts HCl, and 10 parts H_2O.

19.034 PREPARATION OF AUTHENTIC VANILLA EXTRACT

Prep. single fold authentic vanilla ext. with and without added sugar by either USP or Flavoring Extract Manufacturers Association (FEMA) method. Prep. concentrates by evapg single fold ext. on steam bath and dilg with 50% alcohol to appropriate vol.

(**a**) *USP method.*—Cut 100 g vanilla in small pieces, add 200 ml H_2O, and macerate 12 hr in covered container, preferably in warm place. Add 200 ml alcohol, mix well, and macerate ca 3 days. Transfer mixt. to percolator contg 200 g coarse granular sucrose (omit sucrose for prepn without sucrose) and drain. Pack solids firmly and percolate slowly with alcohol (1+1) to obtain total vol. of 1 L.

(**b**) *FEMA method* (*12*).—(Boil all rubber stoppers in 5% NaOH soln and use Tygon tubing for connections.) Prep. laboratory continuous percolator as follows:

Fit 2 hole rubber stopper into neck of custom made Pyrex gas washing bottle made from No. 2962 cylinder, 4.5″ o.d.×12″ long, plain neck opening ca 3″ diam., with coarse porosity fritted glass disk sealed in as close to bottom as possible but above side arm, 9 mm o.d., extending out from side wall between disk and base (available from Corning Glass Co., Corning, N. Y. or H. S. Martin Co., 1916–20 Greenleaf St., Evanston, Ill.). Thru 1 hole place 0–220°F thermometer with bulb at ca center of bottle; thru other hole insert short piece of glass tubing. Attach piece of cheesecloth tied with cord to end of tubing in bottle as strainer and attach other end to Fischer and Porter (Hatboro, Pa.) size 2 flowmeter (tube B2A25, float BSK20, stainless steel float stops, mm scale and flow curve for ml/min., liquid sp. gr. 0.96). Attach upper end of flowmeter with right-angle bend tube to side arm of T-tube with upper end capped with Bunsen valve (rubber tube, closed at one end, with short longitudinal slit) as safety valve.

Attach lower end of T-tube thru Y-tube and 1 hole rubber stoppers to 2 Pyrex tubes (Corning No. 39570, 35 mm diam., with coarse fritted glass disks) in parallel, fitted with milk sediment disks. Unite streams from filters with Y-tube and connect thru glass tube to menstruum reservoir (No. S-8685, 2 L aspirator bottle, E. H. Sargent & Co., 4647 W. Foster, Chicago 30, Ill.) thru 2 hole stopper. Thru other hole fit thermoregulator (No. 17510, nickel plated, Fenwal Inc., Ashland, Mass.) which controls 200 watt custom-made heating mantle fitted to reservoir. Connect outlet of bottle to inlet of stainless steel pump with nipple connections (Model B1, 1/20 hp, Eastern Industries Inc., 100 Skiff St., Hamden, Conn.). Connect outlet of pump to side arm of gas washing extractor bottle. Control pump speed with variable autotransformer to give flow rate of 575 ml/min. and set thermoregulator so thermometer temp. is 120°F.

Charge extractor with 10 oz (dry wt) of cut vanilla and reservoir with 55% alcohol (1136 g alcohol+1090 g H_2O). Percolate 16 hr and remove 25 ml sample for alcohol detn, **19.002.** Calc. g H_2O to add to give 47.5% alcohol (=2790 −132414/% alcohol), add this quantity to reservoir, and continue percolation 24 hr. Remove ext. and adjust to 35% alcohol by adding 36 ml sugar sirup contg 16 g sucrose for each 100 ml ext. (equiv. to 1 lb sucrose/gallon ext.). (Use H_2O for adjustment in prepn without sucrose.)

19.035 PREPARATION OF PAPERS

(**a**) *For small (8×8″) papers.*—Apply no more than 7 samples, including authentics, to single paper. Spot samples equal distance apart. For single strength exts apply four 3 microliter spots at same point, drying between applications with hair dryer, infrared lamp, or other source of mild heat. Do not apply next spot until previous one is dry. For concd exts, dil. to 4 fold concn with 50% alcohol and apply one 3 microliter spot.

(**b**) *For large (22×18″) papers.*—For single strength exts apply four 10 microliter spots at same point with intervening drying as in (**a**). For concd exts, dil. to 4 fold concn with 50% alcohol and apply one 10 microliter spot.

19.036 DEVELOPMENT

Use ascending technic with solvents (**a**) and (**b**). Descending technic may be used with large papers and solvents (**c**), (**d**), and (**e**). Develop small papers 2–3 hr and large papers 12–16 hr, until solvent approaches end of paper. Remove papers from tank and let air dry. Examine under transmitted ultraviolet light and compare fluorescent pattern of spots from samples with those from authentic material.

It is sometimes useful to return dry paper to original solvent and repeat development. Second development may cause greater sepn of some of fluorescent constituents.

LEMON, ORANGE, AND LIME EXTRACTS AND FLAVORS

19.037 Specific Gravity—Official

Det. sp. gr. at 20/20° with pycnometer as in **9.011**.

19.038 Alcohol (*13*)—Official

(Applicable to exts consisting only of oil, alcohol, and water)

Det. sp. gr. at 15.56/15.56° or at 20/20° as in **9.011** and oil content as in **19.048, 19.049,** or **19.101,** and apply following formula: Let S represent sp. gr. of sample; O, sp. gr. of oil; and p, % oil found. Then $100 - p = \%$ H_2O-alcohol soln, sp. gr. of which, represented by P, is calcd as follows:

$$S = [Op + P(100 - p)]/100;$$

therefore

$$P = (100S - Op)/(100 - p).$$

Det. E, alcohol equiv. of P, from **43.021**. It gives % alcohol in alcohol-H_2O soln. To find % alcohol in ext. apply following formula:

$$\% \text{ by vol. of alcohol in ext.} = E(1 - p/100).$$

Value of O for lemon oil may be taken as 0.86 and for orange oil as 0.85.

19.039 Methanol—Official

Pipet 50 ml sample into 200 ml vol. flask, noting temp.; dil. with H_2O to ca 200 ml, and let mixt. stand until oil seps in clear layer at top, or centrifuge and add H_2O to bring lower meniscus to mark. Pour mixt. into dry erlenmeyer contg 5 g *light $MgCO_3$*, stopper, shake well, and filter quickly thru large, dry, folded paper. Place 100 ml aliquot filtrate, measured at same temp., in 300–500 ml distn flask, and add 50 ml H_2O. Attach flask to condenser and distill almost 100 ml. Add H_2O to complete vol. of distillate to 100 ml at same temp., mix well, and proceed as in **9.043** or **9.044**.

Isopropyl Alcohol—Official

Applicable to Lemon Extract in Absence of Acetone (14)

19.040 PREPARATION OF SAMPLE

Place sample contg not >8 g total alcohols (approximation of alc. content may be made from sp. gr. detn and reference to **43.021**), into separator contg in stem cotton pledget wet with H_2O. Add 25 ml 10% NaCl soln and 25 ml petr. ether. Shake well and when layers sep. drain lower layer into flask. Repeat extn with 3 addnl 25 ml portions of the NaCl soln or until alcohol is completely extd. Add H_2O to combined aq. exts until vol. is ca 150 ml. Connect flask to vertical condenser and distill into 100 ml vol. flask, removing flask when distillate is 2–3 ml below mark. Dil. to vol. and mix.

19.041 QUALITATIVE TEST FOR ACETONE

To 2 ml distillate add 0.5 ml *5% alc. o-nitrobenzaldehyde soln* and 1 ml 10% NaOH soln. Mix; then shake with small quantity of $CHCl_3$. Blue color in the $CHCl_3$ indicates presence of acetone.

19.042 DETERMINATION

Pipet 10 ml distillate into 500 ml erlenmeyer contg 50 ml ca $2N$ $K_2Cr_2O_7$ and add 100 ml H_2SO_4 (1+3). Stopper flask, swirl, and let stand 30 min. Add 100 ml 30% $FeSO_4.7H_2O$ soln. Connect flask to vertical condenser thru foam trap. Slowly distill ca 100 ml into 500 ml vol. flask contg 200–300 ml cold H_2O. Dil. to mark, mix, and pipet 25 ml into g-s. flask contg 25 ml $1N$ NaOH; add 50 ml stdzd $0.1N$ I with swirling. Let stand 15 min. Add 26 ml $1N$ HCl and at once titr. residual I with stdzd $0.1N$ $Na_2S_2O_3$, adding starch soln when I color is nearly discharged. Each ml $0.1N$ I consumed in reaction = 1.001 mg isopropyl alcohol.

Applicable to Lemon and Orange Flavors in Presence of Acetone (15)

19.043 APPARATUS

Glassware.—Use foil wrapped stoppers or preferably all-glass still. Provide condenser with adapter which reaches several inches into vol. flask.

19.044 PREPARATION OF SAMPLE

Proceed as in **19.040**, placing 100 ml vol. flask in ice-H_2O bath.

19.045 DETERMINATION OF ACETONE

Pipet aliquot preferably contg 0.1–0.3 g acetone into 100 ml vol. flask and dil. to mark with H_2O. Det. absorbance at 265 mμ with H_2O as reference soln in Beckman DU spectrophotometer or equiv. instrument. Correct for absorbance of H_2O in same cell as used for sample, if necesary. Det. quantity of acetone in the 100 ml vol. flask by reference to std curve prepd from redistd acetone.

In absence of purified acetone, g acetone/100 ml may be estimated from equation: $C = A/3.08$, where C = g acetone/100 ml, A = corrected absorbance in 1 cm cell, and 3.08 = assumed absorbance of 1 g/100 ml soln of acetone in 1 cm cell. Calc. to g acetone/100 ml sample.

19.046 DETERMINATION OF ISOPROPYL ALCOHOL

Proceed as in **19.042**, distg ca 100 ml into 250 ml vol. flask contg ca 100 ml cold H_2O and held in ice-H_2O bath. Dil. to mark with H_2O and det. corrected absorbance as in **19.045**. Det. quantity of acetone in 250 ml vol. flask by reference to std curve prepd as in **19.045**.

In absence of purified acetone, g acetone/250 ml may be estimated from equation: $C' = 2.5A/3.08$, where C' = g acetone/250 ml, 2.5 = diln factor, A and 3.08 are defined in **19.045**. Calc. to g acetone/100 ml sample. Deduct quantity of free acetone as detd in **19.045**, and multiply by 1.035 to obtain g isopropyl alcohol/100 ml sample.

19.047 **Glycerol—First Action**

Proceed as in **11.012** or **11.013**, selecting method according to quantity of sugar present. Use sample contg 0.1–0.4 g glycerol.

Oils of Lemon and Orange in Extracts

19.048 *By Polarization (16)—Official*

Without dilg, polarize sample at 20° in 200 mm tube. Divide reading in °S, **29.020**(a), by 3.2 for lemon ext. and by 5.2 for orange ext. In absence of other optically active substances, result will be % oil by vol. If cane sugar is present, det. as in **19.060** and correct reading accordingly. To obtain % oil by wt from % by vol., multiply vol. % by 0.86 for lemon exts, and by 0.85 for orange exts, and divide results by sp. gr. of original ext.

19.049 *By Precipitation (17)—Official*

Pipet 20 ml sample into Babcock milk bottle, **15.030**(a). Add 1 ml HCl (1+1), then 25–28 ml H_2O previously warmed to 60°. Mix, and let stand in H_2O 5 min. at 60°. Centrifuge 5 min., fill bottle with warm H_2O to bring oil into graduated neck of flask, again centrifuge 2 min., and place flask in H_2O at 60° few min. Note % oil by vol. If >2% oil is present, add 0.4% to % oil noted to correct for solubility of oil. If <2% and >1% is present, add 0.3% for this correction. To obtain % oil by wt from % by vol., multiply vol. % by 0.86 for lemon exts, and by 0.85 for orange exts, and divide result by sp. gr. of original ext.

19.050 *By Precipitation in Presence of Mineral Oil—First Action*

Proceed as in **19.101**.

Oils of Lemon, Orange, or Lime in Oil Base Flavors

By Steam Distillation (18)—Official

19.051 APPARATUS

(**a**) *Steam generator filled with H_2O.*—An oil can holding 1 gallon will serve purpose.

(**b**) *Distillation flask.*—750 ml Kjeldahl flask with short neck; total height ca 10″.

(**c**) *Spray tube.*—Glass tube connected to steam generator; with small perforated bulb at end of tube passing thru rubber stopper and reaching bottom of distn flask.

(**d**) *Bent glass tube.*—Approx. 8 mm diam. Connects distn flask to upright condenser. Shape of this tube allows vapor condensing in tube to return to distn flask.

(**e**) *Liebig condenser.*—With 20″ H_2O jacket.

(**f**) *Wilson receiving flask.*—(Fig. 33.) Babcock test bottle shape with graduated neck but of 250 ml capacity and with vertical glass outlet tube sealed on near bottom. Upper end of outlet tube

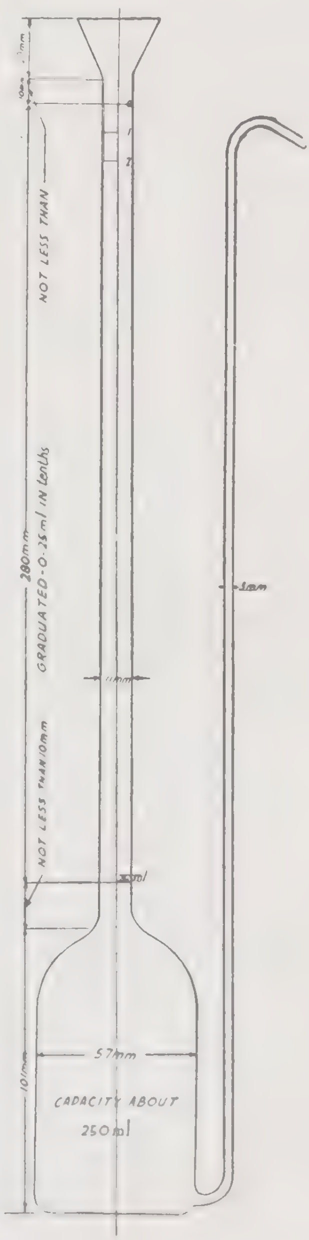

FIG. 33.—WILSON FLASK

is turned down. Neck may consist of portion of buret graduated from 0–25 ml with flared top. Outlet tube is ca 3 mm diam., and end at such height that when flask is filled with H_2O, meniscus in neck will be between 0 and 1 ml marks.

19.052 DETERMINATION

Measure 100 ml sample in graduated cylinder and transfer to distn flask. Immerse flask in H_2O bath and connect to condenser with the bent glass tube. Fill receiving flask with H_2O and so place under condenser that end of condenser is ca 0.5″ above level of H_2O in receiving flask. Place 200 ml graduated cylinder under end of outlet tube to catch displaced liquid. Heat H_2O bath to boiling and pass steam thru sample until 200 ml liquid collects in graduated cylinder.

Disconnect app., let receiving flask stand 15 min., or until sepn of oil is complete, and read vol. of oil in flask. Calc. % (by vol.) of essential oil in sample by dividing reading by 0.90 for lemon oil in corn and cottonseed oils, 0.95 for orange oil in corn and cotton seed oils, and by 0.78 for distd or expressed lime oil in corn and cottonseed oils. Where menstruum is mineral oil, subtract 0.3 ml from reading before dividing by factors 0.90, 0.95, and 0.78 for lemon oil, orange oil, and lime oil, resp.

19.053 *By Polarization (19)—First Action*

Polarize sample at 20° in 200 mm tube, making 5 readings. From av. of readings in °S, **29.020**(a), subtract: for corn oil +0.6°, for cottonseed oil −0.3°, for peanut oil +0.2°, and for mineral oil +5.5°, as correction for rotatory effect of menstruum. To obtain % by vol. of essential oil in mixt., divide corrected polariscopic reading so obtained by factor 3.4 for lemon oil in corn oil, 3.7 for lemon oil in cottonseed oil, 3.6 for lemon oil in peanut oil, 3.5 for lemon oil in mineral oil, 5.4 for orange oil in corn oil, 5.7 for orange oil in cottonseed oil, 5.6 for orange oil in mineral oil, 2.0 for lime oil in corn oil, 2.3 for lime oil in cottonseed oil, and 2.2 for lime oil in mineral oil.

Total Aldehydes (*20*)—Official

19.054 REAGENTS

(a) *Aldehyde-free alcohol.*—Let alcohol, contg 5 g *m*-phenylenediamine hydrochloride/L, stand at least 24 hr with frequent shaking. (Nothing is gained by previous treatment with KOH.) Reflux at least 8 hr, longer if necessary; let stand overnight, and distill, rejecting first 10 and last 5 ml distillate. Store in dark, cool place in well-filled bottles. (25 ml of this alcohol, on standing 20 min. at 14–16° with 20 ml of the fuchsin-bisulfite soln, should develop only faint pink color. If stronger color develops, repeat treatment with *m*-phenylenediamine hydrochloride as above.)

(b) *Fuchsin-bisulfite soln.*—Dissolve 0.5 g fuchsin in 250 ml H_2O, add aq. soln contg 16 g SO_2, let stand until colorless or nearly so, and dil. to 1 L with H_2O. Let stand 12 hr before use and keep in refrigerator. (This soln may deteriorate and should be reasonably fresh when used.)

(c) *Citral std soln.*—Weigh 0.5 g citral into 50 ml vol. flask, dil. to mark with aldehyde-free alcohol at room temp., stopper flask, and mix by shaking. Dil. 10 ml of this soln with aldehyde-free alcohol to 100 ml in vol. flask, stopper flask, and mix by shaking. 1 ml dil. soln = 1 mg citral.

19.055 DETERMINATION

Weigh ca 25 g sample in stoppered weighing flask, transfer to 50 ml vol. flask, and dil. to mark at room temp. with aldehyde-free alcohol. Measure, at room temp., 2 ml (or other suitable quantity) of this soln into comparison tube. Add 25 ml aldehyde-free alcohol (previously cooled to 14–16°), then 20 ml fuchsin-bisulfite soln (also cooled), and dil. to 50 ml mark with aldehyde-free alcohol. Mix thoroly, stopper, and keep 15 min. at 14–16°.

Prep. std for comparison at same time and in same manner, using 2 ml std citral soln, and compare colors developed. Calc. quantity of citral present and repeat detn, using quantity sufficient to give sample ca strength of the std. From this result calc. quantity of citral in sample. If comparisons are made in Nessler tubes, stds contg 1, 1.5, 2, 2.5, 3, 3.5, and 4 mg citral may be prepd and trial comparison made against these, final comparison being made with stds lying between 1.5 and 2.5 mg with 0.25 mg increments.

It is absolutely essential to keep reagents and comparison tubes at required temp., 14–16°. If comparisons are made in a bath (possible only when bath is of glass), use stds within 25 min. after adding fuchsin-bisulfite soln. Give samples and stds identical treatment.

Citral (*21*)—Official
(Lemon and orange exts)

19.056 REAGENT

Metaphenylenediamine hydrochloride-oxalic acid soln.—Remove interfering colored impurities in *m*-phenylenediamine hydrochloride by digesting 3–5 g ca 5 min. with ca 25 ml alcohol, decanting, and repeating 3 times. Dry crystals short time on steam bath. Dissolve 1 g in ca 45 ml 85% alcohol, dissolve 1 g crystd oxalic acid in 45 ml 85% alcohol, and pour 2 solns into 100 ml vol. flask. Add 2 or 3 g fuller's earth, dil. to mark with 85% alcohol, mix, and filter thru double folded paper.

19.057 DETERMINATION

Weigh 25 g sample into 50 ml vol. flask, dil. to mark with alcohol (95% by vol. for exts made

with the oils; 50–95% by vol. for terpeneless exts), and mix. Pipet 2 ml or other suitable quantity of this soln into colorimeter tube, add 10 ml of the reagent, dil. to suitable vol., and compare resulting color with colors of set of stds contg known quantities of std citral soln, **19.054(c)**.

19.058 Total Solids—Official

Proceed as in **9.022**, using 10 ml sample measured at 20°.

19.059 Ash—Official

Ignite residue from 10 ml sample as in **29.012** or **29.013**.

19.060 Sucrose—Official

Neutralize normal wt of sample, evap. to dryness, wash several times with ether, dissolve in H_2O, and proceed as in **29.025, 29.026**, or **29.032**.

LEMON AND ORANGE OILS

19.061 Specific Gravity—Official

Det. sp. gr. at 20/20° with pycnometer as in **9.011**.

19.062 Refractive Index—Official

Use any std instrument, making reading at 20°. *See* **26.009**.

19.063 Optical Rotation—Official

Det. rotation at 20° with any std instrument, 50 mm tube, and Na light. State results in angular degrees on 100 mm basis. If instruments having sugar scale are used, reading for orange oils is above range of scale, but readings may be obtained by use of std levorotatory quartz plates, or by 25 mm tube. (True rotation cannot be obtained by dilg the oil with alcohol and correcting rotation in proportion to the diln.)

19.064 Spectrophotometric Absorbance Characteristics (*22*)—Official

Weigh accurately 1 g (to nearest mg) sample in g-s. weighing bottle. Dissolve in alcohol and transfer quantitatively to 100 ml vol. flask. Dil. to mark, mix well, and pipet 25 ml aliquot into another 100 ml vol. flask, dil. to mark with alcohol, and mix well.

Det. absorbance of the prepd soln in ultraviolet region from 260 to 375 mμ with recording or manual spectrophotometer against alcohol in matched cell. Obtain readings at 5 mμ intervals if manually operated instrument is used. Readings at closer intervals (ca 3 mμ) are preferred between 305–320 mμ. Above 325 mμ readings can be made at intervals of 10 mμ.

If instrument does not read directly in absorbance, A, calc. from % transmittance, T, from tables or from equation: $A = 2 - \log T$. Plot absorbance against wavelength and draw smooth curve thru points.

Correct for background absorbance as follows: Draw straight (base) line AB tangent to curve at point of min. absorbance near 285 mμ (285–295 mμ) and at inflection point where curve levels off at ca 365 mμ (365–370 mμ). Drop vertical line CD from absorption peak (ca 315 mμ) to base line AB. Obtain length of vertical line CD in absorbance units and record as corrected absorbance.

19.065 Physical Constants of 10 Per Cent Distillate (*23*)—Official

Place 50 ml sample in 3 bulb, 120 ml Ladenburg flask having main bulb 6 cm diam. and condensing bulbs, 3.5 cm, 3 cm, and 2.5 cm. Distance from bottom of flask to opening of side arm should be 20 cm. Distill oil at rate of 2 ml/min. until 5 ml distills. Det. refractive index and optical rotation of this distillate as in **19.062** and **19.063**.

Residue after Steam Distillation (*24*)—Official

19.066 APPARATUS

Use steam distillation assembly, Fig. 31, page 236, except use 250 ml distg flask.

19.067 DETERMINATION

Add 50 ml H_2O and 15 ml sample to 250 ml distn flask. Weigh 15 ml of the oil delivered by same pipet to obtain wt sample. Place steam inlet tube in flask, heat contents of flask just to boiling, and connect inlet tube to steam. Adjust flame so that H_2O level remains approx. constant. Steam distill at constant rate of ca 200 ml/hr until 100 ml H_2O collects. Discontinue distn and let flask partially cool; then decant contents of flask into 125–250 ml separator and let it drain.

Rinse flask twice with 15 ml and 8 ml portions alcohol, warming if necessary to dissolve any residue. Pour alcohol rinsings into tared 150 ml beaker. Ext. cooled liquid in separator with 25 ml and 20 ml portions $CHCl_3$. (Add 1–2 drops of HCl (1+2) to separator if there is any tendency for liquids to emulsify.) Add exts to the tared beaker contg alcohol washings; then ext. once with 25 ml ether and add this ext. to others. Evap. exts carefully without spattering on cover of steam bath until ether and $CHCl_3$ are removed. Then evap. residual liquid on open steam bath. Let beaker remain on bath 15 min. after odor of alcohol disappears. Remove, wipe outside of beaker with clean dry cloth, let cool, and weigh. Reheat, cool, and weigh until loss is <2 mg/5 min. heating period. Calc. % residue by steam distn.

Total Aldehydes

19.068 *Fuchsin-Bisulfite Method (21)—Official*

Weigh small quantity of sample into small stoppered flask and dil. with aldehyde-free alcohol in proportion of 2 g lemon oil or 4 g orange oil to 10 ml soln. Det. total aldehydes as in **19.055**, expressing result as citral.

19.069 *Hiltner Method (21)—Official*

Weigh accurately ca 2 g lemon oil or 8 g orange oil into 100 ml vol. flask, dil. to mark with alcohol, and proceed as in **19.057**, using 2 ml of the dil. soln for comparison.

Kleber Method (25)—Official
(For orange oil)

19.070 REAGENT

Phenylhydrazine soln.—Prep. 10% soln in absolute alcohol. Sufficiently pure phenylhydrazine can be obtained by distg commercial product *in vacuo*, rejecting first portions coming over that contain NH_3.

19.071 DETERMINATION

Weigh accurately ca 15 g sample into small, g-s. flask, and add 10 ml phenylhydrazine soln. Let stand 30 min. at room temp. and titr. with 0.5*N* HCl, using Me or Et orange indicator. Titr. similarly 10 ml of the phenylhydrazine soln. Difference in ml 0.5*N* acid used in these 2 titrns ×0.076 = wt citral in sample. If end point is difficult to detect, titr. until soln is distinctly acid, transfer to separator, and drain alc. portion. Wash oil with H_2O, adding washings to alc. soln, back-titr. with 0.5*N* alkali, and make necessary corrections.

Kirsten Modification of the Kleber Method (26)—Official
(For lemon oil)

19.072 REAGENTS

(**a**) *p-Toluenesulfonic acid.*—0.5*N*. Dissolve 95 g *p*-toluenesulfonic acid ($CH_3C_6H_4SO_3H.H_2O$) in absolute alcohol and dil. to 1 L with absolute alcohol. Mix thoroly and filter. Stdze against 0.5*N* NaOH, using Me red.

(**b**) *Methyl yellow indicator.*—Dissolve 0.1 g *p*-dimethylaminoazobenzene in 100 ml absolute alcohol.

19.073 DETERMINATION

Weigh accurately ca 15 g sample into 125 ml g-s. flask and pipet in 10 ml of the phenylhydrazine soln, **19.070.** Let stand 30 min. at room temp. and add 25 ml benzene. Titr. with 0.5*N* *p*-toluenesulfonic acid, using 0.2 ml Me yellow. Titr. similarly 10 ml phenylhydrazine soln. Difference in ml 0.5*N* acid used in 2 titrns ×0.076 = g citral in sample.

Hydroxylamine Method (24)—Official
(For lemon oil)

19.074 REAGENTS

(**a**) *Bromophenol blue indicator.*—Dissolve 0.1 g bromophenol blue in 5 ml 0.05*N* NaOH and dil. to 100 ml with 60% alcohol.

(**b**) *Ethyl orange indicator.*—Dissolve 0.05 g Et orange in 60% alcohol and dil. to 50 ml.

(**c**) *Potassium hydroxide soln.*—0.5*N*. Dissolve 28.05 g KOH in 60% alcohol and dil. to 1 L with same solvent. Stdze against std HCl.

(**d**) *Hydroxylamine soln.*—Dissolve 7.0 g $NH_2OH.HCl$ in 175 ml 60% alcohol. Add either: (1) 0.3 ml bromophenol blue indicator and enough 0.5*N* KOH to give permanent blue color, or (2) 0.3 ml Et orange and enough 0.5*N* KOH to give permanent yellow color. In either case dil. resulting soln to 200 ml with 60% alcohol.

19.075 DETERMINATION

Weigh to nearest 10 mg ca 10 g of the oil into g-s. 50 ml graduate and add 7 ml hydroxylamine soln and 0.1 ml indicator. Shake and neutralize liberated acid with the 0.5*N* KOH to permanent full alk. color of indicator used. Continue shaking and neutralizing until permanent alk. color remains in lower layer after shaking mixt. vigorously 2 min. and letting sepn occur. (Reaction is complete in ca 15 min.) 1 ml 0.5*N* KOH = 0.0761 g citral.

This titrn approximates citral in the oil. Repeat detn as above, using as color std for end point the titrd liquid of first detn, and as vol. of the $NH_2OH.HCl$ soln 1–2 ml more than vol. of the 0.5*N* KOH used in first detn.

Esters (*26*)—Official
(For lemon oil)

19.076 APPARATUS

Expeller.—Prep. rubber stopper with glass inlet and outlet tubes similar to wash bottle. Adjust outlet tube to just reach bottom of centrifuge bottle and place soda-lime tube between inlet tube and source of air.

19.077 REAGENTS

(**a**) *Aldehyde-free iso-amyl alcohol.*—Reflux ca 1 L reagent grade *iso*-amyl alcohol over 35–40 g KOH 60–70 min. Distill in all-glass app., reject first 25 ml distillate, and collect next 850 ml. Store at ca 5°.

(**b**) *Sodium chloride soln.*—Dissolve 160 g NaCl in 500 ml H_2O.

(c) *Carbon dioxide-free water.*—Use freshly boiled and cooled H_2O thruout detn.

19.078 DETERMINATION

Weigh 5 ml oil in beaker or bottle and transfer to 125 ml separator, using exactly 25 ml alcohol to complete transfer. Add 1 ml *50% $NH_2OH.HCl$ soln* and few drops phthln, and mix. Add, from buret or graduated pipet, enough 4% KOH in 80% alcohol to make soln just pink and add drop or so excess. Add 1 drop 20% $NH_2OH.HCl$ soln and shake; pink color should be discharged. Add 25 ml of the *iso*-amyl alcohol and shake. Add 50 ml of the NaCl soln, shake vigorously, let layers sep. (line of division should be sharp), drain, and discard lower layer. Repeat extn with four 30 ml portions of the salt soln and once with 6 ml H_2O, draining and discarding exts each time. Drain remaining *iso*-amyl alcohol-oil layer into 500 ml erlenmeyer. Wash separator once with 25 ml Et alcohol and combine with soln in flask. Add phthln, make liquid just pink with ca 0.2*N* stdzd KOH, and then add from pipet exactly 20 ml of the std KOH in excess.

Reflux soln 45 min. on hot plate; then cool with flask loosely stoppered. Add ca 150 ml H_2O and rotate ca 30 sec., but avoid violent shaking. Transfer liquid into 500 ml separator thru short-stem funnel, rinse flask with 20 ml H_2O, and add to separator. Stopper funnel and let layers sep. Drain lower layer into original flask. Add ca 60 ml H_2O to separator, invert, and rotate to mix; then let layers sep. until most of aq. layer seps. (Small layer of emulsion may remain between layers.)

Drain aq. layer into flask, retaining any emulsion in separator. Keep flask and separator stoppered between addns to avoid contact with air. Add ca 100 ml H_2O to separator, shake vigorously, and drain entire contents into 250 ml centrifuge bottle. Stopper, and centrifuge until 2 well-sepd layers are obtained. Blow off lower layer in centrifuge bottle, using expeller, into flask contg aq. fractions previously sepd, add ca 0.2 ml phthln, and titr., using std 0.2*N* HCl. As end point approaches, repeat addn of indicator and titr. to disappearance of pink color. (Liquid becomes white or grayish.)

Conduct blank detn similarly, using same amounts of all reagents. Subtract titrn of sample from that of blank to obtain the equiv. of 0.2*N* alkali consumed. 1 ml 0.2*N* alkali = 39.2 mg esters as linalyl acetate.

Pinene (*27*)—Official

19.079 *Qualitative Test*

Mix 10% distillate, **19.065**, with 5 ml HOAc, cool mixt. thoroly in freezing bath, and add 10 ml *Et nitrite*. Add slowly with constant stirring, 2 ml HCl (2+1). Keep mixt. in freezing bath 15 min. Collect crystals formed on filter, using suction, and wash with alcohol. Return combined filtrate and washings to freezing bath 15 min. Collect addnl crystals formed on original filter. Wash combined crops of crystals thoroly with alcohol. Dry at room temp. and dissolve in min. quantity of $CHCl_3$. Add MeOH to the $CHCl_3$ soln, little at time, until nitrosochlorides crystallize out. Mount sepd and dried crystals in olive oil and examine under microscope. Pinene nitrosochloride crystals have irregular pyramidal ends, while limonene nitrosochloride crystallizes in needles.

ALMOND EXTRACT

Alcohol—First Action

19.080 *Method I.* (*28*)

Fill 50 ml pycnometer with sample at 15.56°, and empty into separator contg ca 10 g NaCl. Wash out pycnometer several times with satd NaCl, using total of ca 100 ml. Ext. twice with 50 ml portions petr. ether (b.p. 40–60°). Collect petr. ether ext. in second separator and wash with two 25 ml portions satd NaCl soln. Combine original NaCl soln with washings, add little *powd. pumice*, and distill into 100 ml pycnometer (Fig. 18, page 105). When almost 100 ml collects, dil. to mark with H_2O at convenient temp. and det. alcohol from sp. gr. as in **9.013**, using table, **43.021**.

19.081 *Method II.* (*29*)

Det. sp. gr. of ext. at 15.56/15.56° or at 20/20° as in **9.011** and benzaldehyde content as in **19.082.** Apply formula given in **19.038**, using benzaldehyde content as % oil found.

19.082 Benzaldehyde (*30*)—**First Action**

Measure 10 ml sample into each of two 300 ml erlenmeyers and add 10 ml *phenylhydrazine soln* (3 ml HOAc, 40 ml H_2O, 2 ml phenylhydrazine) to one flask and 15 ml to other. Let mixts stand overnight in dark place.

Add 200 ml H_2O and filter thru weighed gooch provided with thin layer of asbestos. Wash ppt first with cold H_2O and finally with 10 ml 10% alcohol. Dry 3 hr at 70° at pressure not >100 mm Hg or to constant wt over H_2SO_4. Wt ppt × 5.408 = wt benzaldehyde in 100 ml sample. If the 2 detns do not agree, repeat operation, using larger quantity phenylhydrazine soln.

19.083 Benzoic Acid (*31*)—**First Action**

Measure 10 ml sample into 100 ml flask and add 10 ml 10% NaOH soln and 20 ml 3% H_2O_2 soln; cover with watch glass and place in H_2O oven.

Oxidation of aldehyde to benzoic acid begins almost immediately; continue heating 5–10 min. after all benzaldehyde odor disappears (20–30 min.).

Remove flask from oven; transfer contents to separator, rinsing off watch glass; add 10 ml H_2SO_4 (1+5); and cool contents of funnel to room temp. under tap. Ext. benzoic acid with 25, 25, 20, and 20 ml portions ether, and wash combined exts with 2 portions of 5–10 ml H_2O, or until all H_2SO_4 is removed. Filter into weighed dish, evap. at room temp., dry overnight in desiccator, and weigh the benzoic acid. Multiply result by 10.

Multiply g/100 ml benzaldehyde obtained in **19.082** by 1.151 to obtain equiv. of benzoic acid and subtract this product from g/100 ml total benzoic acid obtained above. Difference = g benzoic acid/100 ml ext.

Hydrocyanic Acid

19.084 *Qualitative Test—Procedure*

To several ml sample add several drops freshly prepd 3% $FeSO_4.7H_2O$ soln and single drop 1% $FeCl_3.6H_2O$ soln. Mix thoroly and add 10% NaOH soln, dropwise, until no further ppt forms and then H_2SO_4 (1+9) to dissolve ppt. In presence of even small quantities of HCN, Prussian blue coloration or suspension develops.

19.085 *Quantitative Method—Official*
(In absence of chlorides)

Measure 25 ml sample into small flask and add 5 ml *freshly pptd* $Mg(OH)_2$, Cl-free. Titr. with $0.1N$ $AgNO_3$, using K_2CrO_4 as indicator. 1 ml $0.1N$ $AgNO_3$ = 0.0027 g HCN.

Nitrobenzene

19.086 *Qualitative Test—Procedure*

Boil few ml sample with Zn dust and HOAc, and filter. Add 1 drop $CHCl_3$ to filtrate, make strongly alk. with 10% NaOH soln, and heat. Presence of nitrobenzene in original ext. is indicated by development of characteristic odor of phenylisonitrile.

CASSIA, CINNAMON, AND CLOVE EXTRACTS

Alcohol—First Action

19.087 *Method I.—See* **19.080**

19.088 *Method II.* (*29*)

Det. sp. gr. of ext. at 15.56/15.56° or 20/20° as in **9.011**, and oil as in **19.090**, and apply formula given in **19.038**. Use following values for sp. gr. of the oil: Cassia, 1.05; cinnamon, 1.03; and clove, 1.055.

19.089 **Isopropyl Alcohol—Official**

Proceed as in **19.040–19.042.**

19.090 **Oil** (*32*)**—First Action**

Pipet 10 ml sample into Babcock milk test bottle. Remove nearly all alcohol by blowing air into bottle thru small glass tube 30 min., or longer if necessary. Add from 10 ml buret 1 ml *solvent* (equal parts USP mineral oil and H_2O-free kerosene), shake well, and fill with satd $MgSO_4$ soln. Centrifuge 10 min. and read vol. of oil from extreme bottom to extreme top of column. To obtain % oil subtract 5 divisions and multiply remainder by 2.

GINGER EXTRACT

19.091 **Alcohol—First Action—***See* **9.013**

19.092 **Solids** (*33*)**—First Action**

Evap. 10 ml sample nearly to dryness on steam bath, dry 2 hr in oven at temp. of boiling H_2O, and weigh.

19.093 **Ginger (Qualitative Test)** (*34*)**—First Action**

Dil. 10 ml sample to 30 ml, evap. to 20 ml, decant into separator, and ext. with equal vol. ether. Let ether evap. spontaneously in porcelain dish, and to residue add 5 ml 75% H_2SO_4 (by wt) and ca 5 mg *vanillin*. Let stand 15 min. and add equal vol. H_2O. In presence of ginger ext. azure blue color develops.

19.094 **Capsicum (Qualitative Test)** (*35*)**—First Action**

To 10 ml sample add cautiously NaOH soln (1+9) until soln reacts very slightly alk. to litmus paper. Evap. at ca 70° to ca $\frac{1}{4}$ original vol. and make slightly acid to litmus paper with H_2SO_4 (1+9). Transfer to separator, rinsing dish with H_2O, and ext. with equal vol. ether, avoiding formation of emulsion by shaking separator gently 1–2 min. Drain lower layer and wash ether ext. once with ca 10 ml H_2O. Transfer washed ether ext. to small evapg dish, make decidedly alk. with $0.5N$ alc. KOH, and evap. at ca 70° until residue is pasty.

Add ca 20 ml more of the KOH soln and let mixt. stand on steam bath until gingerol is completely saponified (ca 30 min.). Dissolve residue in little H_2O and transfer with H_2O to small separator (vol. should not be >50 ml). Ext. alk. soln with equal vol. ether. Wash ether ext. repeatedly with small quantities of H_2O until no longer alk. to litmus. Transfer washed ext. to small evapg dish and let ether evap. spontaneously. Finally test residue for capsicum by moistening tip of finger, rubbing it on bottom and sides of dish, and then applying finger to end of tongue. Hot,

stinging, or prickly sensation, which persists several min., indicates capsicum or other foreign pungent substances.

PEPPERMINT, SPEARMINT, AND WINTERGREEN EXTRACTS

Alcohol—First Action

19.095 *Method I.—See* **19.080**

19.096 *Method II.* (*27*)

Det. sp. gr. at 15.56/15.56° or at 20/20° as in **9.011**, and oil content as in **19.090**, and apply formula given in **19.038**. Use following values for sp. gr. of the oil: Peppermint, 0.90; spearmint, 0.93; and wintergreen, 1.18.

19.097 Isopropyl Alcohol—Official—*See* **19.040–19.042**

19.098 Oil—First Action—*See* **19.101**

ANISE AND NUTMEG EXTRACTS

Oil (*36*)—First Action

19.099 *Method I.*

To 10 ml sample in Babcock milk test bottle add 1 ml HCl (1+1), then enough half-satd NaCl soln, previously heated to 60°, to fill flask nearly to neck. Stopper and let stand in H_2O at 60° ca 15 min., rotate occasionally, and centrifuge 10 min. at ca 800 rpm. Fill bottle to neck with satd NaCl soln and again centrifuge 10 min. If sepn is not satisfactory or liquid is not clear, cool to ca 10° and centrifuge addnl 10 min. Reading×2 = % oil by vol.

19.100 *Method II.—See* **19.101**

OTHER EXTRACTS AND TOILET PREPARATIONS

19.101 Essential Oil (*37*)—First Action

(Applicable to exts of allspice, anise, caraway, lemon, nutmeg, orange, peppermint, pimiento, rosemary, thyme, wintergreen, and methyl salicylate)

Pipet 10 ml sample (5 ml when oil content is >5% by vol.) into Babcock milk test bottle, add 0.50 ml *solvent* (equal parts USP mineral oil and H_2O-free kerosene) and 1 ml HCl (1+1), and fill to shoulder with satd NaCl soln. Shake bottle 3 min.; then add the NaCl soln to bring column of oil within graduations on neck. Centrifuge 10 min. at high speed and read vol. of oil from extreme bottom to extreme top of column. (Read from extreme bottom to bottom of meniscus at top of column for allspice, peppermint, and pimiento exts.) To obtain % oil subtract 2.5 divisions and multiply remainder by 2. (Multiply by 4 if 5 ml sample is used.)

Essential Oil in Emulsion (*38*)—First Action

19.102 APPARATUS

Use modified oil separator trap, Fig. 34, connected to 500 ml round-bottom flask thru ₮ 24/40 joint, and equipped with tight fitting finger condenser having projection at bottom to facilitate return of the oil to trap.

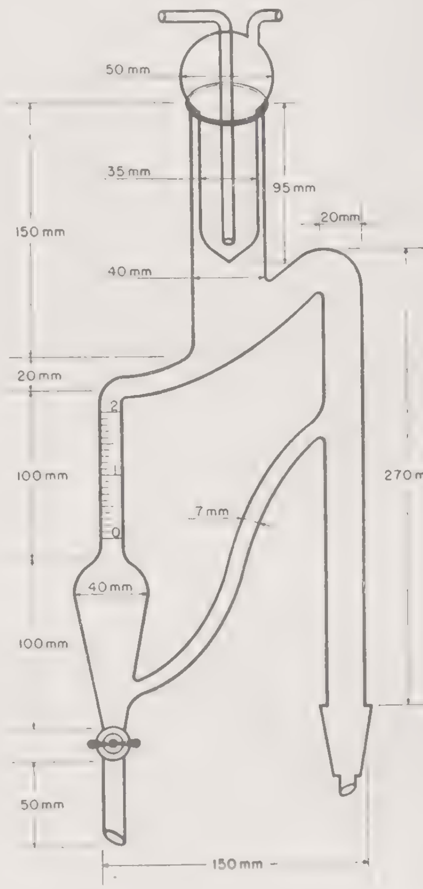

FIG. 34.—OIL SEPARATOR TRAP

19.103 DETERMINATION

Weigh 5–10 ml sample contg not >2 ml essential oil in tared g-s. graduated cylinder. Transfer to the 500 ml flask contg ca 200 ml H_2O, rinsing cylinder by shaking with several 5 ml portions H_2O. Add rinsings to flask.

Fill oil trap with H_2O to overflowing, connect to flask and condenser, and carefully boil 1 hr. Remove heat and let stand several min. Remove enough H_2O from trap to bring oil layer within graduations, let stand 5 min. to complete drainage, and measure quantity of oil from bottom of lower meniscus to highest point of upper meniscus

β-Ionone (*39*)—Official

19.104 *Method I.*

(Applicable to pure solns contg 100 mg or less in 5 ml alcohol)

Place 5 ml alc. sample contg 10–100 mg β-ionone in 125 ml erlenmeyer. Add 95–100 mg solid *m-nitrobenzhydrazide* and dissolve by warming soln on steam bath, taking precautions to prevent loss of alcohol thru evapn. Add 5 ml H_2O, and if soln becomes cloudy, warm until clear. Remove soln from steam bath, add 0.2 ml HOAc, stopper flask lightly, and place on wooden surface to prevent too rapid cooling.

If ca 20 mg or more of β-ionone is present, crystals begin to form within 30 min. after soln reaches room temp. Let stand in room at least 2 hr (overnight does no harm) and add 5 ml H_2O dropwise, mixing soln continuously during addn by rotating flask. Stopper, let stand in room at least 1 hr, and refrigerate overnight (not >48 hr). Filter thru fine fritted glass crucible, wash with 30 ml dil. alcohol (3+7), using wet policeman to remove ppt adhering to flask, and dry at 100°. Wt ppt×0.541 = wt β-ionone. Identify crystals microscopically, **19.107.**

Method II. (*40*)

(Applicable to raspberry concentrates)

19.105 APPARATUS

(**a**) *Steam generator filled with water.*—Oil can holding 1 gallon is convenient.

(**b**) *Distillation flask.*—Round-bottom boiling flask with ₸ 24/40 joint, capacity ca twice vol. of sample to be used.

(**c**) *Still head.*—Adapter, 75° angle, with male connections ₸ 24/40 at bottom and side, and female connection 14/35 at top, with side arm lengthened and bent to fit vertical condenser.

(**d**) *Spray tube.*—Adapter, for use with Woulff bottles equipped with ₸ joints; aeration tube with ₸ 14/35, holes in bulb ca 2 mm diam., length of tubing such that when app. is set up, bulb is situated not >20 mm above bottom of distg flask.

(**e**) *Condenser.*—Coil type with female connection ₸ 24/40 at top with 250–300 mm jacket and outlet tube lengthened to ca 200 mm to reach bottom of 500 ml erlenmeyer receiving flask.

19.106 DETERMINATION

Place 250–1000 ml sample contg not >100 mg β-ionone in distg flask and connect with app. Add enough H_2O to receiving flask to just cover outlet of condenser. Heat sample nearly to boiling on asbestos mat with flame or by immersing it in boiling H_2O bath. As soon as sample reaches temp. of bath or just begins to boil, connect with steam generator and pass rapid current of steam thru sample until ca 500 ml distillate collects.

Add enough H_2O to distillate to reduce alcohol content to ca 10% or less and transfer to large separator. Add 150–200 ml ether, depending upon vol. of soln, so that ca 100 ml is obtained upon sepn. Shake thoroly ca 2 min. Let mixt. settle till clear and drain aq. layer till ca 25 ml remains in separator. Whirl liquid and again let settle.

When clear, drain remainder of aq. layer; then drain ether soln into 125 ml erlenmeyer contg 95–100 mg *m-nitrobenzhydrazide.* After separator drains ca 1 min., close stopcock, pour 10–15 ml ether into separator to wash down sides, let soln settle 1 min., and add to main ether soln. Add 0.2 ml HOAc and dissolve solid reagent by stirring and breaking up lumps with glass rod, warming if necessary to complete soln. Let mixt. stand ca 1 hr and evap. on steam bath to ca 10 ml, passing current of air into flask to hasten evapn and keep down temp.

In meantime make second extn of distillate, using 100 ml ether. Add sepd ether soln to flask contg residue from first ether ext., follow with ether washings of separator, let stand at least 15 min., and evap. to 10 ml as before. Similarly make third extn, using 100 ml ether, add to flask, and evap. as before until 1–3 ml watery liquid and perhaps some oily residue remain.

While flask is still warm, add 5 ml alcohol from pipet, washing down sides of flask, and dissolve residue completely by warming on steam bath, protecting liquid against loss by evapn. Add 5 ml H_2O and warm if necessary to obtain clear soln. Add 0.2 ml HOAc, close with cork stopper, and place flask on wooden surface to prevent too rapid cooling.

After 2 hr, add 5 ml H_2O dropwise, mixing liquid by continuously rotating flask, stopper, and keep at room temp. at least 1 hr (overnight does no harm); then refrigerate overnight (not >48 hr).

Filter on fine fritted glass crucible and wash with ca 30 ml dil. alcohol (3+7). Dry in vac. oven at 70° and weigh. Wt ppt×0.541 = wt β-ionone. Identify crystals microscopically, **19.107.**

If pptd material consists of oily matter mixed with cryst. matter, place fritted glass crucible in gooch holder attached to suction flask. Support test tube with wire within suction flask so as to catch any liquid that passes thru crucible. Add ca 5 ml petr. ether, cover crucible, and let stand ca 5 min. Apply suction just long enough to carry thru any solvent that remains in crucible. Transfer petr. ether soln to small beaker and let evap. spontaneously. Repeat several times until no more sol. matter is obtained by extn. Examine remaining contents of crucible and several resi-

dues microscopically for crystals of β-ionone-m-nitrobenzhydrazide.

19.107 Optical-Crystallographic Properties of β-Ionone-m-Nitro-Benzhydrazide (*41*)—Procedure

This substance in mass is yellowish, but when examined in ordinary light under microscope it is essentially colorless and crystallizes in thin, rod-like plates, many having lath-like or frayed ends, some having 6-side outline. In parallel polarized light (crossed nicols), extinction is parallel and sign of elongation negative. Refractive indices are the min. value, $n_\alpha = 1.548$, invariably shown on elongated fragments when their long dimension is parallel to vibration plane of lower nicol (lengthwise), and max. value, $n_\gamma = 1.648$, usually shown on elongated fragments when their long dimension is at right angles to vibration plane of lower nicol (crosswise).

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20. Fruits and Fruit Products

20.001 Sampling (*1*)—Procedure

(a) *Boxed dried fruit.*—Remove cover, bottom, or one side of box, as convenient. Remove block comprising $\frac{1}{8}$ of contents of box taken from one corner as follows: With sharp knife make vertical cut midway between *ends* of box to center of top surface, extending cut half way to bottom. Make another vertical cut midway between *sides* of box, extending half way to bottom, and continue it until it meets first cut. Remove all fruit included in angle formed by the 2 cuts. Working rapidly, break up lumps, mix thoroly, and take enough sample to fill quart Mason jar, replacing remainder in box. Seal jar and send to laboratory. Sample enough boxes from different parts of pile to constitute at least square root of lot.

(b) *Frozen pack fruit in barrels* (*2*).—Use stainless steel or corrosion-resistant tube ca $1\frac{1}{4}''$ diam. and 36″ long, one end serrated and set to run freely, other end with removable cap and arrangement for use of elec. motor in drilling. To aid in removal of core samples use wooden ram smaller in diam. but longer than tube.

Remove bottom of barrel and take 3 cores evenly spaced around its circumference near chime parallel to and thru full length of barrel. Take fourth core at approx. center of barrel.

(c) *Frozen pack fruit in small containers* (*30–50 lb*) (*2*).—Use modified corrosion-resistant auger 1–$1\frac{1}{2}''$ diam. and 19″ long that can be operated by elec. motor. (Auger should have no lead screw or cutters and angle of face should not be flat but 170–175°.) Collect borings in corrosion-resistant sampling can ca 6″ diam. and 4″ high, open at one end, with outlet at other end ca 1″ long and of diam. slightly larger than that of auger. Place sampling can on surface of frozen fruit and operate auger thru small opening at bottom. Take 3 vertical cores evenly spaced about circumference and ca $\frac{1}{2}''$ from edge of container and take 1 core at or near center. Remove both sampling can and auger simultaneously to prevent borings from falling thru delivery outlet.

20.002 Preparation of Sample—Procedure

Transfer samples received in open packages (*i.e.*, not sterile) without delay to g-s. containers and keep in cool place. To avoid effects of fermentation make prompt detns of alcohol, total and volatile acids, solids, and sugars, particularly in case of fruit juices and fresh fruits. (Portions for detn of sucrose and reducing sugars may be weighed and kept several days without fermenting if the slight excess of neutral $Pb(OAc)_2$ soln required in detn is added.) Prep. various products for analysis as follows:

(a) *Juices.*—Mix thoroly by shaking to insure uniform sample, and filter thru absorbent cotton or rapid paper. Prep. fresh juices by pressing well-pulped fruit and filtering. Express juice of citrus fruits by one of common devices used for squeezing oranges or lemons, and filter.

(b) *Jellies and sirups.*—Mix thoroly to insure uniform sample. Prep. soln by weighing 300 g thoroly mixed sample into 2 L flask and dissolve in H_2O, heating on steam bath if necessary. Apply as little heat as possible to minimize inversion of sucrose. Cool, dil. to mark, mix thoroly by shaking, and use aliquots for the various detns. If insol. material is present, mix thoroly and filter before taking aliquots.

(c) *Fresh fruits, dried fruits, preserves, jams, and marmalades.*—Pulp by passing thru food chopper, or by use of high speed blender, Hobart mixer, or other suitable mechanical mixing app., or by grinding in large mortar, and mixing thoroly, completing operation as quickly as possible to avoid loss of moisture. With dried fruits, pass sample thru food chopper 3 times, mixing thoroly after each grinding. Set burrs or blades of food chopper as closely as possible without crushing seeds. Grind entire contents of No. 10 or smaller container. Mix contents of larger containers thoroly by stirring and remove portion for grinding. With stone fruits, remove pits and det. their proportion in weighed sample.

Prep. soln by weighing into 1.5–2 L beaker 300 g sample, well pulped and mixed in high speed blender or other suitable type of mechanical grinder; add ca 800 ml H_2O; and boil 1 hr, replacing at intervals H_2O lost by evapn. Transfer to 2 L vol. flask, cool, dil. to vol., and filter. With unsweetened fruit ashing is facilitated by addn of sugar before boiling; therefore weigh 150 g fruit, add 150 g sugar and 800 ml H_2O, and proceed as above.

(d) *Canned fruits.*—*See* **30.001**. Carefully invert by hand all fruits having cups or cavities if they fall on sieve with cups or cavities up. Cups or

cavities in soft products may be drained by tilting sieve, but no other handling of these products while draining is permissible. Examination of sirup in which fruits are preserved is often sufficient. Sep. liquor by draining, **30.001**, and treat as in (**a**).

Fill of Container of Frozen Fruits (*3*)—First Action

20.003 APPARATUS

(**a**) *Overflow can.*—With device for lowering frozen fruit into liquid and for removing it, Fig. 35. Can is ca 8″ diam. and ca 9″ high with overflow spout of $\frac{3}{16}$″ i.d. ($\frac{1}{4}$″ o.d.) Cu tubing. Solder tubing to opening on side of can ca $\frac{1}{2}$″ from bottom and bend upward parallel to side of can to ca 2″ below top where it is bent away and downward to form inverted U. Form spout by cutting tubing on outer side of U where it makes ca 45° angle with can, making cut parallel to bottom of can. Opening of spout is ca $\frac{1}{8}$″ below lower surface of U-bend. Bend end of spout up or down until overflow, caused by adding excess of liquid to can, will end abruptly. (Proper adjustment of tube and addn of enough liquid will secure this effect.) Lowering device consists of $\frac{1}{2}$″ metal frame $5\frac{3}{4}$″ square contg $\frac{1}{2}$″ mesh screening attached on one side to perpendicular handle 12″ long bent outward at top.

(**b**) *Plastic bags.*—Pliable at 0°F; capable of holding vac.; ca 8×10″ when flat. (Cry-O-Rap bags, Type L, Dewey & Almy Chemical Co., Lockport, N. Y., or equiv.)

(**c**) *Freezer or cold room.*—At or near 0°F.

(**d**) *Refined light mineral oil such as odorless kerosene.*

20.004 DETERMINATION

Transfer frozen sample from container and inner wrapper, if any, to plastic bag. Remove excess air from bag by inserting glass tube attached to vac. line. Twist bag top to close, hold twist with pinch clamp, and trim off loose end. Pretest bags to be certain they will not leak.

Place overflow can in freezing compartment and fill can, in which lifting device is inserted, with light mineral oil at temp. of freezing compartment (ca 0°F). Add enough excess mineral oil (ca 300 ml) to produce siphon effect in overflow, collecting overflow in beaker. Place empty, calibrated graduated cylinder under overflow tube and immerse frozen fruit sample completely in the mineral oil, using lifting device. Record vol. of overflow in cylinder to nearest ml. Correct this vol. for displacement of empty plastic bag and pinch clamp (ca 7 ml) to obtain net displacement of the frozen fruit. Redet. displacement of sample to check reproducibility of procedure.

For packages with square corners, calc. H_2O capacity of outer container by multiplying inside length, width, and height in cm. For packages with curved edges or irregular shape, det. H_2O capacity as follows: Place empty container in beaker or pan contg enough H_2O to reach to within 1 cm of top of container when it is resting on bottom of beaker or pan. Note that no air is trapped by bottom of container. Add H_2O from calibrated 500 ml buret to fill container to capacity, or to measured headspace if indented top has been removed. Read H_2O capacity directly from buret.

Det. % fill of container by dividing net displacement of frozen fruit by H_2O capacity of outer container and multiply by 100.

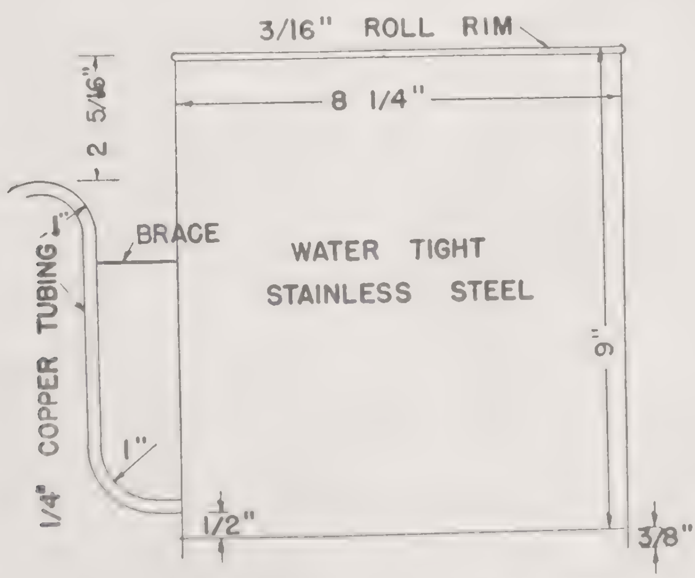

FIG. 35.—APPARATUS FOR DETERMINING VOLUME OF FROZEN FRUITS BY DISPLACEMENT

20.005 Drained Weight of Frozen Fruits (*4*)—First Action

After obtaining gross wts, immerse packages in H_2O agitated and maintained at $20 \pm 1°$. (If packages are not H_2O-tight, place in suitable plastic bag, remove excess air by use of vac., and tie off.) Avoid agitation of packages during thawing by using clamps or weights if necessary. When center of packages reach bath temp. as detd by preliminary experiments (ca 2–3 hr for 10.5–16 oz. containers), remove from bath, blot off adhering H_2O, and open with min. agitation.

Tare No. 8 sieve with light-wt drip pan. Use 8″ diam. sieve if container holds <3 lb, 12″ if more. With screen tilted and supported for drainage, distribute contents of package evenly over screen in one sweeping motion. After 2 min. from time drainage begins, transfer sieve with fruit to drip pan and weigh. Obtain net wt of packages by subtracting wt empty containers from their gross wts.

20.006 Approximate Fruit Content of Fruit-Sugar Mixtures (*5*)—Procedure

Let sample thaw and come to room temp. in original container. Mix sample thoroly in high speed blender. Filter portion of sample thru strong lens paper or other suitable medium. Det. refractometer reading, **29.011**, correct to 20°, and report as % sol. solids (sucrose). Calc. % fruit, X, from equation: $X = (100 - M)\ 100/(100 - F)$, where M is sol. solids (as sucrose) of the fruit-sugar mixt. and F is sol. solids of fruit ingredient in the mixt. if known; otherwise use av. sol. solids of authentic fruits (*6*).

20.007 Alcohol—Official

Det. alcohol in 50 g original material as in **11.004.**

20.008 Moisture in Dried Fruits (*7*)—Official

Spread 5–10 g prepd sample, **20.002(c)**, as evenly as possible over bottom of metal dish ca 8.5 cm diam. provided with tight-fit cover, weigh, and dry 6 hr at 70° under pressure not >100 mm Hg. (Metal dish must be in direct contact with metal shelf of oven.) During drying admit to oven slow current of air (ca 2 bubbles/sec.) dried by passing thru H_2SO_4. Replace cover, cool dish in desiccator, and weigh. Disregard any temporary drop in oven temp. during early part of drying period owing to rapid evapn of H_2O. With raisins, and other fruit rich in sugar, use ca 5 g sample and dry and weigh in dish with ca 2 g finely divided asbestos. Moisten with hot H_2O, mix sample and asbestos thoroly, evap. barely to dryness on steam bath and complete drying as above.

Total Solids—Official

20.009 *Insoluble Matter Present*

Fresh and canned fruits, jams, marmalades, and preserves.—Weigh accurately into large flat-bottom dish, 20 g pulped fresh fruit, or quantity of fruit products that will give not >3–4 g dry material. If necessary to secure thin layer of the material, add few ml H_2O and mix thoroly. Dry at 70° under pressure not >100 mm Hg until consecutive weighings made at 2 hr intervals do not vary >3 mg.

20.010 *Insoluble Matter Absent*

Fruit juices, jellies, and sirups.—Proceed as in **29.007, 29.008, 29.009, 29.010,** or **29.011,** using sample prepd as in **20.002(a)** or **(b)**.

Water-Insoluble Solids (*8*)—First Action

20.011 *Method I.*

For use with büchner, prep. filtering medium consisting of either circular disk of absorbent cotton ca 80 mm diam., weighing ca 1.5 g, or coarse, qual. filter paper (7–15 cm diam., Whatman No. 4 or 41-H or equiv.). For use with 60° funnel, prep. absorbent cotton circle ca 12.5 cm diam. weighing ca 2 g, or 12.5 cm filter paper. Wash filtering medium with hot H_2O, and dry overnight at 100–110° in open, flat-bottom Al dish of suitable size provided with tight-fit cover. Cool closed dish and contents 1 hr in desiccator and weigh to nearest mg.

Weigh 25 or 50 g well-mixed sample, **20.002(c)**, to nearest 10 mg, transfer to 400 ml beaker, dil. to ca 200 ml mark with hot H_2O, mix, and boil gently 15–20 min., occasionally replacing H_2O lost by evapn. Filter by gravity thru the prepd cotton or paper, and keep H_2O-insol. solids from forming closely adhering mat on surface of filtering medium by frequent addns of portions of sample. Wash with ca 800 ml hot H_2O, loosening H_2O-insol. solids from filter with each addn. Remove excess H_2O from cotton by gently squeezing it on 60° funnel, or by application of suction on büchner. Transfer to original weighing dish, and wipe off any remaining portions of H_2O-insol. solids on filter or funnel with previously weighed portion of prepd filtering medium. Dry overnight at 100–110°, cool 1 hr in desiccator, and weigh.

Method II. (Rapid Method)

20.012 APPARATUS

(a) *Weighing dishes.*—Al or tinned Fe, 5¼″ diam. × ¾″ high, with tight-fit cover (16 mm film

holders obtainable from camera stores; Al dishes weigh ca 40 g, tinned Fe ca 85–90 g).

(**b**) *Rapid drying device.*—Moisture Teller, model 271T, manufactured by Harry W. Dietert Co., 9330 Roselawn Ave., Detroit 4, Mich., or force-draft drying oven set at 100°.

20.013 DETERMINATION

Fit 15 cm filter paper (Whatman No. 4 or 41-H, or equiv.) into 12.5 cm büchner, add half of 7 cm paper (used to wipe any insol. solids from büchner after filtration and washing sample), wash with boiling H_2O, apply suction, and dry, using Moisture Teller and pan or force-draft oven. Transfer to weighing dish, cool, and weigh, using tare consisting of weighing dish and paper. (Approx. time of drying, 5 min. at $102 \pm 3°$.)

Weigh 25 or 50 g well-mixed sample (high speed blender) to nearest 10 mg, transfer with hot H_2O to 400 ml beaker, adjust to ca 200 ml with hot H_2O, stir, and boil gently few min. Place prepd filter in büchner; attach to suction flask, but do not attach flask to suction line. Pour 50–100 ml boiling H_2O on filter, and when steady flow of H_2O passes thru filter, transfer sample to filter, portionwise if necessary. Wash insol. solids with boiling H_2O and collect 850–900 ml filtrate. During washing, keep solids from forming tight mat on surface by portionwise addns of boiling H_2O. When washing is finished, apply suction and aspirate thoroly. Transfer paper and H_2O-insol. solids to Moisture Teller pan, using extra piece of weighed filter paper to complete transfer, and dry at $102 \pm 3°$ ca 15 min., depending on quantity of H_2O-insol. solids. After drying, transfer sample to weighing dish, cool in desiccator, and weigh. (Wt H_2O-insol. solids/wt sample) $\times 100 = \%$ H_2O-insol. solids.

20.014 Seeds in Berry Fruits (*9*)—First Action

Prep. sample by thoro mixing, using high speed blender. Transfer 50 ± 0.01 g with ca 500 ml hot H_2O to blender and mix 1–2 min. Transfer mixt. to No. 20 screen and use addnl hot H_2O to transfer and wash bare seeds (hot H_2O from tap is suitable). Transfer seeds on screen to Al dish, previously weighed, with tight-fit cover (readily accomplished by transferring to 7 cm Whatman No. 4 paper previously dried and weighed with the dish, in Coors 2A büchner). Dry at 100° in force-draft oven 30 min. and weigh. To det. av. wt of one seed, count out and weigh separately several 100-unit lots. Report av. wt of one seed in mg and number of seeds/100 g sample. After detn of H_2O-insol. solids of sample, calc. and report % of total due to bare seeds and % due to non-seed H_2O-insol. solids.

20.015 Soluble Solids (By Refractometer) in Fresh and Canned Fruits, Jams, Marmalades, and Preserves (*10*)—First Action

(Insol. matter present)

Proceed as in **29.011.** % sol. solids = % solids detd by refractometer $\times (100 - b)/100$, where $b = \%$ H_2O-insol. solids.

NOTE: U. S. Federal std for jams and preserves makes no correction for H_2O-insol. solids.

20.016 Ash (*11*)—Official

Proceed as in **29.012** or **29.013,** ashing at not $>525°$, using 25 g juices, fresh fruits, or canned fruits, and 10 g jellies, sirups, preserves, jams, marmalades, or dried fruits.

If ash of H_2O-sol. portion only is desired, evap. on steam bath to dryness 100 ml prepd soln, **20.002**(**b**) or (**c**). Proceed as in **29.012** or **29.013.**

20.017 Alkalinity of Ash—Official

Introduce measured excess of 0.1*N* HCl into Pt dish contg ash obtained in **20.016**, warm on steam bath, cool, add few drops Me orange, and titr. excess acid with 0.1*N* NaOH. Report as alky, number of ml 0.1*N* acid required to neutralize ash from 100 g sample, and as alky number, number of ml 1*N* acid required to neutralize 1 g ash. Reserve soln for detn of S in ash.

Potassium (*12*)—Official

20.018 ASHING OF SAMPLE

(**a**) *Slow ashing.*—Ash 15–30 g sample (representing ca 15 g fruit) as in **20.016.**

(**b**) *Rapid ashing.*—To 15–30 g sample in Ni or Pt dish (preferably flat-bottom, $3\frac{1}{2}''$ diam., and 1″ high) add 1 ml 25% $Mg(NO_3)_2.6H_2O$ soln; evap., char, and heat at temp. not $>550°$ until C-free (ca 15 min.). Cover dish with watch glass and cool.

Chloroplatinate Methods

20.019 REAGENTS

(**a**) *Chloroplatinic acid soln.*—Dissolve 4.4 g H_2PtCl_6 (contains 2.1 g Pt) in H_2O and dil. to 100 ml. 1 ml of this soln ppts ca 10 mg K_2O. Use ca 20% excess.

(**b**) *Calcium carbonate suspension.*—Mix 50 ml alcohol with 50 ml glycerol and add 50 g powd. $CaCO_3$. Keep in dropping bottle and shake vigorously before use.

(**c**) *Calcium formate suspension.*—Mix 50 ml alcohol with 50 ml glycerol and add 50 g fine crystals $Ca(CHO_2)_2$. Keep in dropping bottle and shake vigorously before use.

(**d**) *Alcoholic sodium hydroxide and sodium formate soln.*—Shake NaOH pellets with alcohol contg 0.2 ml formic acid/100 ml until satd.

20.020 PREPARATION OF ASH SOLUTION

Wet down ash, **20.018**(a) or (**b**), with 5–10 ml H_2O, cover dish with watch glass, and acidify with slight excess of HCl (1+4) (2–3 ml for **20.018**(a) and 4–5 ml for **20.018**(**b**)).

20.021 DETERMINATION

(**a**) *Gravimetric chloroplatinate method.*—Rinse watch glass into dish and evap. ash soln to dryness on steam bath. Add 5 drops HCl (1+1) to residue. Add 5–10 ml hot H_2O and rub sides and bottom of container with policeman. Transfer ash soln to 250 ml beaker with 50–75 ml hot H_2O, add few glass beads, and heat to boiling. Make distinctly alk. with NH_4OH and add enough satd $(NH_4)_2C_2O_4$ soln for complete pptn (usually not >1 ml), cover beaker, and heat until ppt becomes granular enough to filter readily (incipient boiling 30 min. usually suffices). Filter thru 5 or 7 cm fine texture paper into large Pt dish and wash thoroly with hot H_2O (5–6 fillings of filter usually suffice).

Evap. soln nearly to dryness on steam bath and add 1 ml H_2SO_4 (1+1). So rotate dish that H_2SO_4 comes in contact with all residue, adding little H_2O if necessary. Return dish to steam bath and evap. all H_2O possible at that temp. Then heat dish, preferably on hot plate, at ca 150° until bubbling caused by decomposition of oxalates ceases, and gradually increase temp. until H_2SO_4 evaps. (When properly controlled, this treatment takes 45–90 min.) Heat sample cautiously over burner, being careful to avoid loss due to sputtering during decomposition of NH_4 compounds. Finally heat dish to redness to remove traces of NH_4 compounds and complete ignition. Cool, and add 5 drops HCl (1+1) to residue.

Transfer ash soln to 100–200 ml round-bottom porcelain dish, using ca 50 ml hot H_2O. Add small excess of the H_2PtCl_6 soln. Place mixt. on steam bath and rotate dish from time to time to prevent ppt from baking on side of dish, and evap. to paste. (It is advisable to start evapn with several steam bath rings removed, and as concn progresses to replace rings so that heat is applied only to surface of dish covered by liquid.) Avoid exposure to NH_3 fumes at all times.

Add ca 50 ml 90% alcohol to dish and transfer to gooch with asbestos mat, or 30 ml gooch with medium porosity fritted disk. Wash 8 or 10 times with 20 ml portions 90% alcohol; then 5 or 6 times with 10 ml portions NH_4Cl soln, **2.059**(a). Again wash well 6 or 8 times with 20 ml portions 90% alcohol.

Dry ca 30 min. in 100° oven, cool, and weigh. Wash the K_2PtCl_6 thru gooch with hot H_2O, using slight suction; then wash gooch with alcohol, dry, cool, and weigh. Difference in wt$\times$0.1938 = K_2O. Report results as mg/100 g original sample.

(**b**) *Short gravimetric chloroplatinate method.*—Proceed as in (a), pars. 3–5.

(**c**) *Short volumetric chloroplatinate method.*—Proceed as in (a), par. 3–4 only. Then dissolve ppt in gooch with several portions of boiling H_2O. Stir gently to facilitate soln, and using suction, collect filtrate and washings in 250 ml wide-mouth, lipped erlenmeyer. Add 1 ml formic acid, heat to boiling, and simmer ca 2 min. after metallic Pt forms. Add 10 ml HNO_3 (1+1), mix, and add small excess 0.1N $AgNO_3$, accurately measured. Boil vigorously 5 min., cool, and filter thru gooch with medium porosity fritted disk. Wash ppt 5 or 6 times with 2% HNO_3, breaking up lumps with glass rod, and collect filtrate and washings in erlenmeyer. Add 5 ml satd Fe alum indicator, **4.015**(**e**), and with vigorous agitation titr. excess $AgNO_3$ with 0.1N NH_4CNS to first definite end point.

(**d**) *Long volumetric chloroplatinate method.*—Transfer ash soln to 100–200 ml round-bottom porcelain dish, using hot H_2O. If C or other insol. material remains in ash, filter into dish thru 5.5 cm medium texture paper, washing metal dish and filter 4 or 5 times with 5 ml portions hot H_2O. Evap. (rapidly if desired) to 10–15 ml, add H_2PtCl_6 soln in excess, and evap. on steam bath to heavy consistency (impinge stream of air on surface of liquid to hasten evapn, and rotate dish from time to time to wash crystals into center). Cool dish, and if crystals become dry on cooling, add drop HCl (1+4) and 2 drops H_2O, so that mass remains moist and holds salts in soln. Add ca 10 ml 90% alcohol, triturate with policeman, and decant immediately onto prepd gooch (15 ml gooch with rapid filtering mat, ca 3 mm thick, of acid-washed, long-fiber asbestos, to which is added 0.5–1 ml $CaCO_3$ suspension in such manner that asbestos is completely covered when liquid is removed by suction).

(Same asbestos pad may be used for 3 or 4 detns. Top surface, impregnated with Pt powder, may be removed with sharp wire when filtering becomes too slow, and thin layer of asbestos may be added from time to time if pad becomes thin. Asbestos should be completely covered with layer of $CaCO_3$ before each filtration.)

Wash dish and crystals once or twice more with ca 5 ml portions 90% alcohol; then transfer ppt to gooch. Wash crucible free of H_2PtCl_6 with 90% alcohol; then wash 3 or 4 times with 5 ml portions NH_4Cl, pouring soln gently into crucible from graduate so that $CaCO_3$ mat is not disturbed. Wash NH_4Cl from crucible with 4 or 5 washings 80% alcohol. Cover K_2PtCl_6 completely with the $Ca(CHO_2)_2$ by adding ca 1 ml of the suspension. Remove liquid with suction and wash once with

alcohol. Cover with Na_2CO_3 to depth of 2–3 mm and moisten with ca 1 ml alc. NaOH-$NaCHO_2$ soln. Ignite 5–10 min. at ca 500°. (If furnace is not available, ignition is conveniently conducted as follows: Prep. air bath by suspending nichrome triangle ca 1″ from bottom of metal crucible, ca 2.5″ diam. at top and 3″ deep, and placing inverted porcelain crucible cover on triangle. Heat over Meker burner with flame so adjusted that inside of crucible is just red as far up as suspended cover.)

Cool gooch, add ca 5 ml hot H_2O, and filter by suction into 400–500 ml g-s. erlenmeyer. Repeat addn of hot H_2O once or twice to remove Na_2CO_3. With suction on, add ca 5 ml HNO_3 (1+1) dropwise to decompose $CaCO_3$ and Na_2CO_3. Wash several times with hot H_2O.

Add 10 ml HNO_3 (1+1) to flask. Cool, and add 5 ml Fe alum soln, **4.015**(e), and quantity of 0.1*N* $AgNO_3$ soln, accurately measured, greater than that necessary to ppt Cl. Dil. to ca 200 ml with H_2O, add 1–2 ml *nitrobenzene*, stopper flask, and shake vigorously ca 30 sec. to coagulate AgCl. Titr. excess $AgNO_3$ with 0.1*N* NH_4CNS. 1 ml 0.1*N* $AgNO_3$ = 8.1 mg K_2PtCl_6, 1.57 mg K_2O, or 1.3 mg K.

If approx. $AgNO_3$ requirement is unknown, following procedure is recommended: After adding the Fe alum soln, note reading on NH_4CNS buret and add few drops to flask. Then, while swirling flask, add 0.1*N* $AgNO_3$ from buret until soln is milk white, after which add addnl 1 or 2 ml. Continue detn as directed previously, beginning "Dil. to ca 200 ml . . ." Back-titr. with the NH_4CNS soln and include vol. added before addn of $AgNO_3$ in calcg $AgNO_3$ equiv. of sample.

Gravimetric Cobaltinitrite Method

20.022 REAGENTS

(a) *Trisodium cobaltinitrite soln.*—Prep. aq. soln contg 2.0 g Na cobaltinitrite in each 10 ml and test to insure that it gives recovery of 98–102% with 20 mg quantities K_2O. Filter before use and prep. fresh soln before each set of detns.

(b) *Nitric acid solns.*—Approx. 1*N*, 0.1*N*, and 0.01*N*.

(c) *Nitric acid-dipotassium sodium cobaltinitrite wash soln.*—Sat. portions of the 0.01*N* HNO_3 with few mg $K_2NaCo(NO_2)_6.H_2O$ by shaking (ca 1 hr). Filter thru Pyrex fine fritted glass crucible or equiv.

20.023 DETERMINATION

Add enough 1*N* HNO_3 to ash, **20.018**(a) or (b), in Pt dish to yield excess of ca 2 ml of the acid in the 20 ml soln used in pptn (ca 3 ml for **20.018**(a) and 5 ml for **20.018**(b)). Wash into 25 ml vol. flask, dil. to vol., and mix. Let stand at least 1 hr and filter, if necessary, thru small paper. Withdraw 10 or 20 ml aliquot (3–35 mg K_2O), adjust to 20 ml with 0.1*N* HNO_3 if necessary, and cool to ca 20°.

Add from pipet, while stirring, 10 ml of the Na cobaltinitrite soln cooled to 20°. In range 3–18 mg K_2O (most preserves) add reagent dropwise with stirring; in range 18–35 mg (most fruits) add reagent in steady stream from fairly rapid delivery pipet (20–22 sec.). Let stand 2 hr at ca 20°. Protect from laboratory fumes. Filter thru tared fine fritted glass crucible (Pyrex, 30 ml capacity, is convenient), using the cobaltinitrite wash soln to make transfer.

Wash ppt 9 times with at least 4 ml portions of wash soln, once with 2 ml 0.01*N* HNO_3, and 5 times with 2 ml portions alcohol, releasing vac. each time before adding washing fluid. Aspirate until apparently dry. Dry 1 hr at 100°, cool in desiccator, and weigh. Formula of ppt is $K_2NaCo(NO_2)_6.H_2O$, and mg ppt × 0.2074 × 100/g sample in aliquot = mg K_2O/100 g sample.

NOTES: Pyrex F or Jena 1G4 porosity crucibles or equiv. are acceptable and can be used number of times before cleaning with hot 5% H_2SO_4. Final wash with 0.01*N* HNO_3 should be restricted to 2 ml. $K_2NaCo(NO_2)_6$ ppt obtained in K detns is suitable for satg the wash soln. Control sample of pure dry KCl should be run from time to time. Stock soln of 2 mg K_2O/ml is convenient. Adjust to 20 ml, using 2 ml 1*N* HNO_3 for acidification.

Manganese (*13*)—First Action

20.024 PREPARATION OF SOLUTION

Dissolve ash in HCl (1+2), evap. to dryness, and heat 1 hr at 110° to dehydrate any SiO_2. Dissolve residue in HCl (1+4) and filter into vol. flask. Wash filter thoroly and dil. to vol.

20.025 DETERMINATION

To aliquot of prepd soln add enough Br-H_2O to oxidize any ferrous Fe to ferric state. Boil off excess Br. Dil. to 150 ml and heat to boiling. Add enough 10% $NaH_2PO_4.H_2O$ soln to combine with all the Fe and Al. Add plenty of bromocresol green indicator, **2.093**(e), and while mixt. is gently boiling add freshly prepd 10% NaOH soln dropwise to first permanent turbidity or initial color change in event no Fe or Al is present. Continue neutralization by slowly adding 20% NaOAc soln to give yellow-green color. (Fe and Al phosphates are completely pptd at pH 4, where bromocresol green indicator is yellow-green.) Boil gently 1–2 min. if any ppt of Al or Fe phosphate forms. Let settle, filter, wash carefully, and discard ppt.

To filtrate add 10 ml of the NaOAc soln and adjust pH to 4.2–4.4 (indicated by yellow-green color with bromocresol green indicator) by adding HCl (1+5) dropwise. Add enough Br-H_2O to color

soln distinctly orange, cover with watch glass, and boil gently ca 3 min., taking great care to avoid bumping. Let mixt. settle, add little more Br-H_2O, and again boil gently 1–2 min. Again let settle, filter, and wash beaker and filter thoroly. (Reserve filtrate for Ca and Mg detns.)

Dissolve hydrated oxide ppt from filter in original beaker with as little *satd SO_2 soln* as possible. Wash paper thoroly with hot H_2O. Boil to remove all odor of SO_2, add 10 ml H_2SO_4 and 10–20 ml HNO_3, carefully dil. to 50–75 ml, and heat to boiling, slowly introducing small quantities of KIO_4 (ca 0.05 g) with spatula until max. color is produced (ca 0.2 g KIO_4). Cool, and transfer to vol. flask. (Mn in final diln for colorimetric comparison should be not >1 mg/50 ml.) Compare color with stds prepd as in **6.014,** except substitute 10 ml HNO_3 for the $Fe(NO_3)_3$ soln. % Mn_3O_4 = ml $KMnO_4 \times 0.4826$.

Calcium (*14*)—First Action

20.026 *Double Precipitation Method*

Evap. filtrate from Mn detn, **20.025,** to 100–150 ml. Boil off any Br remaining and adjust pH to 4.4–4.6 (green to green-blue with bromocresol green, **2.093(e)**) by adding 20% NaOAc soln (pH 4.4–4.6 is most favorable for pptn of Ca oxalate). Add enough satd Na oxalate soln dropwise to ppt all Ca from boiling soln, and continue to boil until ppt begins to settle, or digest 15 min. on steam bath. Let settle until clear, filter, and wash ppt thoroly with hot H_2O. Reserve filtrate and washings for Mg detn.

Carefully wash ppt back into original beaker, heat, and dissolve oxalate by adding as little HCl as possible. Reppt Ca by adding NH_4OH soln (1+9) dropwise until pH is again 4.4–4.6 (green to green-blue with bromocresol green). Add slight excess of satd NH_4 oxalate soln while still hot. Digest on steam bath 1 hr and set aside until supernatant is clear, preferably overnight. Filter and wash with hot H_2O. Det. Ca either gravimetrically as in **7.023** or volumetrically as in **6.011** or **6.012** (for small quantities gravimetric method is preferred). Report as CaO.

If Mg is not to be detd, ppt Ca once from boiling soln (freed from Fe, Al, and Mn) with satd NH_4 oxalate soln, and proceed as above, beginning "Digest on steam bath 1 hr . . . "

20.027 *Single Precipitation Method*

Evap. filtrate and washings from Mn detn, **20.025,** to 200–250 ml. Add 8–10 drops bromocresol green indicator and enough 20% NaOAc soln to change pH to 4.8–5.0 (blue). Cover with watch glass and heat to boiling. Ppt Ca slowly by adding *3% oxalic acid soln*, 1 drop every 3–5 sec., until pH is changed back to 4.4–4.6 (optimum for Ca oxalate pptn) as indicated by appearance of distinct green. (Change of color indicates excess of oxalic acid—more would develop yellow tints, showing undesirable displacement of pH.) Boil 1–2 min. and let settle until clear. Filter and wash thoroly with hot H_2O. Det. either gravimetrically or volumetrically as in **20.026.**

20.028 **Magnesium** (*15*)—**First Action**

Add 2–3 drops HCl to filtrate and washings from Ca detn, **20.026,** and evap. to 75–100 ml. If quantity of phosphates naturally in sample, or added for purpose of pptg Fe and Al, is not enough to ppt all Mg expected, add more but avoid large excess. For this purpose neutralize with NH_4OH (1+9) until permanent ppt forms and add enough 10% $NaH_2PO_4.H_2O$ soln to ppt all Mg present. Dissolve ppt by slowly adding HCl (1+9) dropwise. Use as little HCl as possible to obtain complete soln.

Use care and patience in next step because $MgHPO_4$ begins to ppt at pH 6.7–6.8, which is critical point. Heat soln to gentle boiling and add NH_4OH soln (1+9) at rate of 4 drops/min. while maintaining gentle boil until cryst. ppt begins to form. (First ppt must be cryst., not gelatinous. If first ppt is gelatinous, redissolve with little HCl and start pptn again more slowly. Stirring assists crystn, but sides of beaker should not be scratched. After crystals have formed in considerable numbers, hasten pptn. This treatment gives cryst. $MgHPO_4$.) Continue adding the dil. NH_4OH until soln is slightly ammoniacal. Let mixt. cool slightly; then add $\frac{1}{3}$ the vol. of NH_4OH slowly and with constant stirring. Let stand until ppt is converted to $MgNH_4PO_4$, preferably overnight.

Filter and wash carefully with the dil. NH_4OH until Cl-free. Dry and ignite slowly until C-free. Cover and ignite intensely. Weigh white $Mg_2P_2O_7$ and report as MgO. $Mg_2P_2O_7 \times 0.3623$ = MgO. (Ignition of dark colored residues with 1 drop 20% NH_4NO_3 soln often improves color. If nitrate is added, use care to avoid spattering.)

Phosphorus

Volumetric Method (16)—Official

20.029 REAGENTS

(a) *Molybdate soln.*—(1) Mix thoroly 50 g MoO_3 (99.5–100%) and 140 ml H_2O and dissolve by addn of 72 ml NH_4OH with stirring; (2) dissolve 50 g powd. tartaric acid in 140 ml H_2O; (3) mix 295 ml colorless HNO_3 with 400 ml H_2O. When solns are cool, pour soln (1) into soln (2) with stirring, and then pour combined solns into soln

(3). Keep in warm place (ca 40°) overnight, filter thru asbestos, and store in bottle with loosely stoppered, plastic screw cap. When free from phosphates, soln is practically colorless.

(**b**) *Ammonium nitrate soln.*—Dissolve 500 g NH_4NO_3 in H_2O and dil. to 1 L.

(**c**) *Carbon dioxide-free water.*—Recently boiled and cooled H_2O.

(**d**) *Sodium hydroxide and hydrochloric acid std solns.*—0.1N. Prep. as in **42.030–42.034** and **42.009–42.011**.

20.030 DETERMINATION

Dissolve ash, **20.016,** in 10–15 ml H_2O and 3 or 4 ml HCl and evap. to dryness on steam bath. Take up in 10 ml hot HCl (1+9) and transfer to 300 ml erlenmeyer, keeping vol. to ca 50–60 ml. (If P_2O_5 is likely to be >10 mg, take aliquot.) Add 20 ml of the NH_4NO_3 soln and heat in H_2O bath to 45–50°. Add 20 ml of the freshly filtered molybdate soln (this quantity will ppt up to 20 mg P_2O_5) and let flasks remain in bath 30 min. at 45–50°, swirling contents at ca 5 min. intervals. To prevent tipping, weight flask with lead rings or by other means.

For filtration use filter-tube (so-called carbon filter), ca 28 mm i.d., fitted with removable, perforated porcelain disk from Caldwell crucible. (Caldwell crucible or gooch may also be used.) Prep. quick filtering pad 2/16–3/16″ thick, using short-fiber asbestos. For convenience in washing and in transferring filter tubes, provide suction flask with rubber stopper having hole somewhat larger than stem of filter tube.

With full suction, filter ppt and wash flask and then filter tube with ca 6 portions cold H_2O, using 150–200 ml total. Test for complete washing by passing 25 ml CO_2-free H_2O thru flask and filter tube into clean suction flask. Immediately disconnect suction and add 1 drop each of 0.1N NaOH and phthln, which should yield strong pink color.

Loosen pad and porcelain disk with wire or narrow rod inserted in stem end, and transfer to flask. Place filter tube in neck of flask, dissolve any ppt on walls with the std alkali, and rinse down filter tube with ca 25 ml of the CO_2-free H_2O. Add enough std alkali to dissolve ppt. Stopper flask, swirl, and let stand, mixing from time to time, until yellow ppt completely dissolves. Dil. to ca 75 ml with the CO_2-free H_2O, add 10 drops phthln, and titr. with the std acid to complete disappearance of pink color, matching end point with another flask contg H_2O and asbestos only. If alkali adheres to fragments of asbestos, making end point uncertain, add slight excess of the acid and complete titrn with the std alkali. 1 ml 0.1N NaOH = 0.3086 mg P_2O_5. Subtract alkali consumed in blank detn.

Colorimetric Method (17)—Official

20.031 REAGENTS

(**a**) *Molybdenum blue soln.*—Place 9.78 g MoO_3 (99.5–100%) in 500 ml Kjeldahl flask, add ca 150 ml H_2SO_4 (36±0.5N), and heat with gentle mixing until dissolved. Cool to 150°. Weigh, on small watch glass, 0.440 g very finely powd. Mo metal (99.5–100%) and transfer to Kjeldahl flask by sliding watch glass down neck of flask. Keep at 140–150° and mix vigorously until Mo is dissolved (some larger particles may remain). Cool, transfer to 250 ml vol. flask, rinse Kjeldahl with H_2SO_4, and transfer rinsings to vol. flask. Finally fill flask to 250 ml with H_2SO_4 and mix well. Dil. 10 ml of this reagent with H_2O and titr. with 0.1N $KMnO_4$ to pink color that persists 1 min. (reagent should be 0.110±0.001N; if <0.109N add calcd quantity of Mo and dissolve by reheating in Kjeldahl flask to 150°). Preserve the deep green soln in g-s. bottles, carefully avoiding *all* contamination.

(**b**) *Dilute molybdenum blue soln.*—With pipet previously wet inside with H_2O, pipet 10 ml (**a**) into ca 60 ml H_2O in 100 ml vol. flask. Rinse pipet into flask, mix, cool, dil. to mark with H_2O, and mix. Use within 8–10 hr of prepn.

(**c**) *Sodium hydroxide soln.*—3.60±0.05N. Should contain not >0.0005% PO_4. Dissolve the NaOH in H_2O, using As-free Pyrex or porcelain vessel, cool, and titr. with std acid. Preserve in paraffin-lined container. Avoid leaving this reagent in glass equipment for any extended period.

(**d**) *Normal sodium hydroxide.*—From (**c**) prep. ca 1N NaOH. Preserve in As-free Pyrex or paraffin-lined container fitted with 1 hole stopper bearing Pyrex medicine dropper.

(**e**) *Sodium alizarin sulfonate soln.*—Dissolve 0.20 g Na alizarin monosulfonate in 100 ml H_2O and filter. Preserve in indicator bottle.

(**f**) *Phosphate std soln.*—0.05 mg P_2O_5/ml. Dissolve 0.1917 g pure dry KH_2PO_4 in ca 200 ml H_2O and add 10 ml ca 1N H_2SO_4 and 6 drops 0.1N $KMnO_4$. Dil. to exactly 2 L. This soln keeps indefinitely in well-stoppered Pyrex bottle.

(**g**) *Glass beads.*—Boil supply of small glass beads (2 or 3 mm diam.) in aqua regia, wash clean with H_2O, and dry.

20.032 PREPARATION OF SAMPLE

Transfer portion of sample contg 0.5–2.5 mg P_2O_5 to 500 ml Kjeldahl flask. (For detn of P_2O_5 on H_2O-sol. portion of fruits or fruit juices, 25 or 30 ml (equiv. to 3.75 or 4.5 g fruit) of sample soln prepd as in **20.002**(**a**) or (**c**) is convenient aliquot. For jams and jellies 50 ml prepd soln, **20.002**(**b**) or (**c**), may be taken. If sample has low fruit content, take larger aliquot.)

Add 5 ml H_2SO_4 from pipet or buret; then add 10 ml HNO_3 and 5 or 6 glass beads. Place flask on digestion rack over free flame. Protect flask from flame by intervening asbestos mat with hole of such size that surface of H_2SO_4 is above mat. Boil over moderate flame until darkening begins (avoid excessive charring). Add few ml HNO_3 and again boil until slight darkening begins or until SO_3 fumes are evolved from clear colorless or amber soln. (In case of jams or jellies, 3 or 4 addns (ca 5 ml each) of HNO_3 may be necessary.) Add 0.5 ml 60% $HClO_4$ to hot flask and continue fuming few min. (To avoid violent explosions of $HClO_4$ in presence of org. matter do not add >0.5 ml at one time and then only after practically all org. matter has been removed with HNO_3; *do not fail to take all precautions advised in use of* $HClO_4$.) When digest is colorless or very slightly greenish-yellow, cool somewhat, cautiously add 50 ml H_2O, and boil to fumes to remove traces of HNO_3. Cool, add ca 25 ml H_2O, transfer to 100 ml vol. flask, mix, cool, dil. to vol., and mix thoroly.

20.033 DETERMINATION

Transfer 20 ml aliquot of sample digest and 0, 2, 4, 6, 8, 10, and 12 ml of the std phosphate soln to 100 ml vol. flasks (Kohlrausch sugar flasks are convenient) marked at 70 ml. To stds add 30 ml ca $1N$ H_2SO_4. To samples add 20 or 25 ml H_2O, and to all flasks add 3 drops of the Na alizarin sulfonate soln and then exactly 10 ml of the $3.6N$ NaOH soln. Adjust acidity to just yellow with the $1N$ H_2SO_4 and $1N$ NaOH until single drop of acid just changes color of soln to yellow. Dil. to 70 ml and mix by swirling. Place flasks in boiling H_2O bath and bring to that temp. With pipet add exactly 10 ml of the dil. Mo blue reagent, directing stream into soln (do not let it run down side of flask), mix by swirling, and continue to heat in boiling H_2O bath exactly 20 min. Cool rapidly in cold H_2O, dil. to vol., and mix.

Keep stds and unknowns at same temp. by immersing flasks in boiling H_2O bath in which H_2O comes above level of soln in flask. (Simple H_2O bath may be prepd by placing ½" mesh wire screen in bottom of 12 or 14" pan and filling with H_2O to such depth that liquid in flasks is below level of H_2O. Place pan on stand and heat with large Meker burner with flame so adjusted that it spreads over bottom of pan and keeps entire contents at gentle rolling boil. Place flasks only around edge of pan and weight with Pb rings or otherwise support to prevent tipping. Keep bath at rolling boil throughout heating period and add *boiling* H_2O to bath as needed to keep level of H_2O above level of liquid in flasks. Keep thermometer in bath and do not permit variation of >2° between center and edge of pan.

Det. absorbance at 650 mμ with suitable photometer (neutral wedge photometer (*18*) with 1" cell and No. 65 filter is convenient).

This method covers range up to 0.6 mg P_2O_5 in final 100 ml soln. Make large scale graph of stds, plotting mg P_2O_5 against instrument readings. (Graph paper 20×36" with 10 lines/inch is convenient.) From graph convert sample readings to mg P_2O_5/100 ml final soln. If preferred, equation of line may be calcd as described by Klein and Vorhes (*19*) and this equation used in conversion.

NOTES

Instrument need be calibrated only once for each batch of reagents provided adjustment is not altered and temp. of boiling H_2O bath remains same. It is advisable, however, to develop 1 or 2 stds with each batch of unknowns to detect possible change of conditions.

Stdzn under these conditions automatically corrects for blank on reagents, except HNO_3 and $HClO_4$. These reagents have not been found to contain significant quantities of As or P. It is well, however, to det. digestion blank on these reagents from time to time.

In analysis of heterogeneous samples, such as lots of fresh fruit, for total P_2O_5, it may be necessary to digest larger portion than specified to minimize sampling and weighing error. In that case it is convenient to take double size sample and double quantity of H_2SO_4 (10 ml), dil. digest to 200 ml, and finally transfer 20 ml aliquot to 100 ml vol. flask for color development. Quantity of sample digested may be varied to suit nature of sample if final aliquot taken for color development contains not >1 ml H_2SO_4 and not >0.6 mg P_2O_5.

Fe, nitrate, and As interfere in color development. Nitrates are not present in solns prepd as described, and neither Fe nor As is ordinarily present in fruit or fruit products in sufficient quantity to interfere. If presence of excessive As or Fe is suspected, their interference may be prevented by procedure used by Zinzadze (*20*). Proceed as above to point, "Adjust acidity to just yellow . . . " after which add 10 ml exactly $1N$ H_2SO_4 and then 10 ml 8 per cent Na_2SO_3 soln, and dil. to 70 ml. Heat in boiling H_2O bath 1 hr. Then again refer to previous directions and continue with "add exactly 10 ml of the dil. Mo blue reagent . . . " Stds and blank must then be treated in exactly same manner.

20.034 Sulfur in Ash—Official

(For products contg a basic ash)

Add 5 ml HCl (1+2.5) to soln after detn of alky of ash, **20.017**, and evap. to dryness. Heat 1 hr at 110° to dehydrate any SiO_2. Take up in 5 ml of the HCl and filter, washing paper well with hot H_2O. Heat filtrate to boiling and add dropwise from buret or pipet 5 ml 10% $BaCl_2.2H_2O$ soln. Evap. to 100 ml and let stand overnight.

Filter on weighed gooch or Munroe crucible or on 7 cm ashless paper, wash with hot H_2O until filtrate is Cl-free, dry, ignite over Bunsen burner, and weigh as $BaSO_4$. As quantity of ppt is small

exercise great care and make detn in duplicate. Report result as mg S/100 g.

20.035 Total Sulfur (*21*)—**First Action**

(For sulfured products and for samples contg little ash or acidic ash)

In largest available casserole that fits in elec. muffle furnace, place 1–3 g MgO (1 g for fruit juices, 3 g for heavily sugared products and for dried fruits) or equiv. quantity of $Mg(NO_3)_2 .6H_2O$ (6.4 or 19.2 g), 1 g powd. sucrose, and 50 ml HNO_3. Add 5–10 g prepd sample, **20.002**(**a**), (**b**), (**c**), or (**d**). Place same quantities of reagents in another casserole for blank. Evap. on steam bath to paste. Place casserole in cold elec. muffle and gradually heat (not >525°) until all NO_2 fumes are driven off. (All org. matter will have been destroyed.)

Cool, dissolve, and neutralize with HCl (1+2.5), adding excess of ca 5 ml. Filter, heat to boiling, and add dropwise 5 ml 10% $BaCl_2.2H_2O$ soln. Evap. to 100 ml, let stand overnight, filter, wash, ignite, and weigh the $BaSO_4$. Correct result for $BaSO_4$ obtained in blank and report as mg S/100 g. (Detn should be made in room free from S fumes.)

20.036 Total Chlorine (*22*)—**First Action**
See **6.065–6.068**

20.037 Alcohol Precipitate (*23*)—**First Action**

To 100 ml prepd soln, **20.002**(**b**) or (**c**), in beaker, add 4–8 g sucrose (1 or 2 lumps cube sugar) if sugar is not already present, and evap. to 20–25 ml. If H_2O-insol. matter seps during evapn add more sugar. Cool to room temp. and add, slowly and with constant stirring, 200 ml alcohol. Let stand at least 1 hr, filter on 15 cm qual. paper, and wash ppt with alcohol. Do not permit alcohol ppt to dry before transferring from paper.

Wash ppt back into original beaker with hot H_2O, rinsing paper thoroly. Evap. soln to ca 20 ml and add 5 ml HCl (1+2.5). If H_2O-insol. matter seps, stir well and, if necessary, warm slightly to dissolve. Again ppt with 200 ml alcohol, let stand 1 hr, and filter thru paper. Wash ppt and paper thoroly with alcohol to remove all HCl. Rinse ppt from paper into Pt dish with hot H_2O, evap. to dryness on steam bath, dry to constant wt in oven at 100°, and weigh; ignite and reweigh. Loss in wt is alcohol ppt.

As ppt in many samples is colorless and almost invisible, take care that none is lost in dissolving and transferring operations. If quantity of alcohol ppt, indicated by its vol. in first pptn, is not excessive, the second filtration may be made thru gooch contg thin asbestos mat. If alcohol ppt is very pure and small in quantity it may not be visible at first; in this case add small quantity of electrolyte, like NaCl, to flocculate alcohol ppt and render it visible.

20.038 Pectic Acid (*24*)—**First Action**

Transfer 200 ml aliquot prepd soln, **20.002**(**b**) or (**c**), to beaker, add 8–12 g sucrose (2 or 3 lumps cube sugar) if soln does not already contain sugar, and evap. to ca 25 ml. If org. acids are to be detd in filtrate from the pectin, cool, add 3 ml $1N$ H_2SO_4, and immediately add 200 ml alcohol with constant stirring. Let ppt formed settle, filter on 15 cm qual. paper, and wash with alcohol. If org. acids are not to be detd, omit addn of H_2SO_4.

Transfer ppt to original beaker with hot H_2O, evap. to ca 40 ml, and cool to 25° or below. If H_2O-insol. matter seps during evapn, stir vigorously, and if necessary add few drops HCl (1+2.5), and warm; then cool again. Dil. 2–5 ml 10% NaOH soln, depending on vol. ppt, to 50 ml, and add to soln of the alcohol ppt. Let stand 15 min., add 40 ml H_2O and 10 ml HCl (1+2.5), and boil 5 min. Filter and wash ppt of pectic acid with hot H_2O. (This filtration should be rapid and filtrate clear. If filtrate is cloudy or of colloidal nature, reject detn. Colloidal filtrates are due to insufficient alkali or to saponification at too high temp., or both. In such cases, repeat detn, using more alkali and keeping temp. low.)

Wash ppt of pectic acid back into beaker, adjust to vol. of 40 ml, cool to <25°, and repeat saponification with the dil. NaOH soln, pptn with the dil. HCl, and boiling as above. Again filter and wash ppt of pectic acid with hot H_2O, but only to point where test of filtrate shows negligible quantity of acid. (Not >500 ml total filtrate should be necessary.) Wash the pectic acid into Pt dish, and dry on steam bath and finally in oven at 100° to constant wt. Weigh, ignite, and reweigh. Loss in wt = pectic acid.

20.039 Protein—Official

Proceed as in **2.036**, using 5 g jelly or other fruit product contg large quantity of sugar, or 10 g juice or fresh fruit, and larger quantity of the H_2SO_4 if necessary for complete digestion. % $N \times 6.25$ = % protein.

Titratable Acidity (*25*)

20.040 *Indicator Method*

(**a**) *Colorless or slightly colored solns—Official.*—Dil. to ca 250 ml, with neutralized or recently boiled H_2O, 10 g prepd juice, **20.002**(**a**), or 25 ml prepd soln, **20.002**(**b**) or (**c**). Titr. with $0.1N$ alkali, using 0.3 ml phthln for each 100 ml soln being titrd. Report as ml $0.1N$ alkali/100 g or 100 ml original material.

(**b**) *Highly colored solns.—First Action.*—Dil. sample of known wt with neutralized H_2O and titr. to just before end point with 0.1*N* alkali, using 0.3 ml phthln for each 100 ml soln being titrd. Transfer measured quantity (2 or 3 ml) of soln into ca 20 ml neutral H_2O in small beaker. (In this extra diln, color of fruit juice becomes so pale that phthln color is easily seen.) If tests shows that end point is not reached, pour extra dild portion back into original soln, add more alkali, and continue titrn to end point. By comparing dilns in small beakers, differences produced by few drops 0.1*N* alkali can be easily observed.

Glass Electrode Method—First Action

20.041 DETERMINATION

Before use, check electrometer and glass electrode with std buffer solns, **42.007–42.008.** Rinse glass electrode in H_2O several times until reading is ca pH 6. Where electrode construction requires, drain few ml satd KCl soln from bridge during washing. Immerse electrode and bridge in sample contained in beaker. (Sample should titr. 10–50 ml 0.1*N* NaOH and be contained in initial vol. of 100–200 ml.) Stir moderately. Set electrometer for ca pH 6 and add the alkali quite rapidly until deflection of meter is small upon momentarily closing circuit. Set electrometer dial for pH 7 and add alkali slowly. After pH 7 is reached, finish titrn by adding the 0.1*N* alkali 4 drops at time, and record total vol. and pH reading after each addn. (Add whole drops, so that fraction of drop does not remain on buret tip.) Continue titrn at least 4 drops beyond pH 8.1, and interpolate data for titrn corresponding to pH 8.1. pH values used for interpolation should lie in range 8.10 ± 0.2.

Notes:

(1) Always keep glass electrode covered with H_2O when not in use.

(2) If strongly acid cleaning solns are used, electrode requires several hr to come to equilibrium on standing in H_2O.

(3) If electrode, bridge-arm, and stirrer are wiped lightly with piece of filter paper previous to insertion into std buffer, same soln may be used for several checks on instrument.

(4) With some electrometers it is necessary to recheck elec. balance with std cell frequently during extended titrns. pH should also be checked after each of first few detns or until batteries come to nearly constant rate of discharge, indicated by no further need for adjustment.

(5) When unshielded leads are used on electrode, air-driven stirrer is necessary to eliminate elec. fields. Extension leads may be made of ordinary, insulated, braided Cu wire.

20.042 Volatile Acidity—Official

Dissolve 10 g sample, dil. to 25 ml, and steam distill as in **11.030.** 1 ml 0.1*N* alkali = 0.0060 g HOAc.

Total Tartaric Acid *(26)*

Bitartrate Method—Official

20.043 APPARATUS

Device for filtering at 0°.—Use app. similar to that described in **16.017(d)**.

20.044 REMOVAL OF PECTIN

Take sample prepd as in **20.002** with titratable acidity ca 3 ml 1*N* acid and solids content not >20 g. Designate as *A*, ml 1*N* alkali required to neutralize sample. Adjust vol. sample to ca 35 ml by evapn or by addn of H_2O, add 3 ml 1*N* H_2SO_4, and heat to 50°. Transfer adjusted sample to 250 ml vol. flask, rinse with 10 ml hot H_2O, and finally with alcohol; cool, dil. to mark with alcohol, shake, and let stand until pptd pectin seps, leaving clear liquid, overnight if necessary. Transfer to centrifuge bottle, add 0.2 g filter-aid, shake vigorously, centrifuge, and decant thru retentive paper (cover funnel with watch glass). Pipet 200 ml filtrate into centrifuge bottle.

If sample contains alcohol, esters of organic acids may be present, and saponification is necessary. Adjust vol. to 35 ml, add $A + 3$ ml 1*N* KOH, heat to ca 60°, and let stand overnight. Add $A + 6$ ml 1*N* H_2SO_4, transfer to 250 ml vol. flask, and proceed as above.

20.045 DETERMINATION

To soln in centrifuge bottle add vol. $Pb(OAc)_2$ soln, **20.046(c)**, equal to $A + 3$ ml, or in case saponification was made, $A + 6$ ml, and 0.2 g filter-aid; shake vigorously 2 min. and centrifuge. Test supernatant with few drops of the $Pb(OAc)_2$ soln and if ppt forms, add more of the $Pb(OAc)_2$ soln, shake, and again centrifuge. Decant and let drain thoroly by inverting bottle several min. To material in centrifuge bottle add 50 ml 80% alcohol, shake vigorously to disperse ppt, add 150 ml more 80% alcohol, shake, centrifuge, decant, and drain.

To Pb salts in centrifuge bottle add ca 150 ml H_2O, shake thoroly, and pass in H_2S to satn. Unsatn is indicated by presence of partial vac. obtained by stoppering bottle, shaking, and observing partial vac. when carefully removing stopper. Transfer to 250 ml vol. flask, dil. to mark with H_2O, and filter thru folded paper. Transfer 100 ml clear filtrate to 250 ml I flask, tared with 2 or 3 glass beads. (Harvard trip balance sensitive to 0.1 g is convenient.) Evap. on gauze over flame to ca 30 ml, remove from flame, add second 100 ml aliquot, and evap. to 19 ± 0.5 g. Neutralize with 30% KOH soln, 1 drop at time, using phthln, and add *one* drop of the alkali in excess. Add 2 ml HOAc, 0.2 g filter-aid (Celite 545 is satisfactory), and slowly, with agitation, add 80 ml 95% alcohol. Cool in cracked ice-salt mixt.,

shake vigorously 2 min., place in refrigerator, and hold overnight at 0°.

Cover filtering disk, **20.043,** with thin layer of asbestos and place over it thin layer of filter-aid. Place cracked ice in outer funnel, wash filter mat with ice-cold alcohol, and let stand few min. to cool filter thoroly. Swirl flask to suspend filter-aid and ppt, and filter at 0°, sucking mat dry. (Use filtrates and washings for *l*-malic acid detn.) Wash stopper with ca 15 ml ice-cold 80% alcohol, letting wash liquid run into pptn flask. Stopper and shake to wash flask well. Stirring rod bent at 45° angle 1″ from end helps in washing inside of filter tube. Conduct wash liquid completely around inside of filter tube and suck dry. Wash flask and filter tube with two 15 ml portions ice-cold 80% alcohol. While filtering, keep flask cold with cracked ice. Remove ice from outer funnel and transfer ppt and pad to pptn flask with boiling CO_2-free H_2O. Heat almost to boiling and titr. with $0.1N$ alkali, using phthln. 1 ml $0.1N$ alkali $=0.015$ g tartaric acid. Tartaric acid/0.64 = tartaric acid in sample taken.

Citric Acid

Pentabromacetone Method (27)—Official

20.046 REAGENTS

(**a**) *Potassium permanganate soln.*—Dissolve 5 g $KMnO_4$ in H_2O and dil. to 100 ml.

(**b**) *Ferrous sulfate soln.*—Dissolve 200 g $FeSO_4.7H_2O$ in H_2O, dil. to 500 ml with H_2O, and add 5 ml H_2SO_4.

(**c**) *Lead acetate soln.*—Dissolve 75 g normal $Pb(OAc)_2$ in H_2O, add 1 ml HOAc, and dil. to 250 ml.

20.047 REMOVAL OF PECTIN

Measure or weigh accurately desired quantity of prepd sample, **20.002,** with titratable acidity ca 3 ml $1N$ acid and solids content not >20 g, into 250 ml vol. flask and add H_2O to make total vol. 70 ml. Add 2 ml $1N$ HNO_3 to liberate acids and heat to 50°. Dil. nearly to neck of flask with alcohol and cool to room temp. Dil. to mark with alcohol, mix, filter on funnel lined with cotton, and collect at least 220 ml filtrate. (Toward end, filtration is slow; by gathering ends of the cotton and squeezing enclosed residue, desired quantity of filtrate may be secured.)

20.048 ISOLATION OF POLYBASIC ACIDS

Det. titer, t, of 10 ml of the alc. filtrate in terms of ml $0.1N$ NaOH, using phthln.

Pipet 200 ml of the alc. soln into 400 ml beaker, add $(2t+2)$ ml $1N$ NaOH, and place on steam bath 30 min. Cool mixt. to room temp., add 5 ml $1N$ HOAc, and rinse with alcohol into 250 ml centrifuge bottle. Add $0.6t$ g finely powd. $Pb(OAc)_2$. (Quantity $0.6t$, derived from $0.03t(200/10)$, expresses g $Pb(OAc)_2$ required to form the Pb salts of the acids in the 200 ml soln. Quantity indicated is greater than necessary by factor of 1.5 and is generally sufficient.) Shake vigorously 5 min., add 0.2 g Filter-Cel, fill bottle with alcohol, and mix thoroly. Centrifuge and add few drops $Pb(OAc)_2$ soln to supernatant. If ppt forms within 1 min., add more $Pb(OAc)_2$ and repeat centrifuging. Decant and discard supernatant. Completely disperse Pb salts by adding portions of 80% alcohol and shaking.

Fill bottle with 80% alcohol, mix thoroly, and centrifuge. Discard liquid and repeat washing with 80% alcohol. Disperse Pb salts in 50 ml H_2O, dil. to vol. of 150 ml, and sat. with H_2S. Shake 1 min. and rinse into 250 ml vol. flask. Dil. to mark and filter thru large fluted paper, *pouring back until bright.*

20.049 DETERMINATION

Evap. 200 ml of the isolated acid soln, **20.048,** to ca 20 ml, rinse into 250–300 ml tared, g-s. erlenmeyer, and adjust with H_2O to net weight of ca 40 g. Add 2 g KBr and 5 ml H_2SO_4, and, if necessary, heat to ca 50° and let stand 5 min. Add 20 ml of the $KMnO_4$ soln from pipet or buret slowly (1–2 ml portions), swirling flask few sec. after each addn. Let stand undisturbed 5 min. and cool to 15°. Add $FeSO_4$ soln slowly with constant agitation until mixt. starts to clear. Shake 1 min., continue addn of the $FeSO_4$ soln until MnO_2 is dissolved, and add few ml excess. Add 20 g anhyd. Na_2SO_4, with accompanying swirling to assure soln (if Na_2SO_4 remains substantially undissolved, repeat detn). Cool to 15° and shake vigorously 5 min.

Immediately, while still cold, collect the pentabromacetone on asbestos in gooch and wash residual ppt from flask with portion of filtrate. Finally wash crucible with 50 ml cold H_2O and let crucible remain under suction few min. Dry crucible overnight in H_2SO_4 desiccator and weigh, or place crucible in drying train and aerate until loss in wt does not exceed few tenths mg, making first weighing after 20 min.

Remove pentabromacetone from crucible with alcohol followed by ether, filling crucible 3 times with each solvent. Dry crucible 10 min. at 100°, cool in desiccator, and weigh. Difference in the 2 wts = wt pentabromacetone. Calc. g anhyd. citric acid from formula: $X=0.424P$, where $X=$ g citric acid in aliquot; $P=$ g pentabromacetone; and $0.424=$ theoretical factor for converting pentabromacetone to anhyd. citric acid. Anhyd. citric acid in sample taken for analysis $=X/0.64$.

For drying the pentabromacetone by aspiration, use app. shown in Fig. 36, where A is gooch, 28 mm diam., loosely packed with cotton; B is

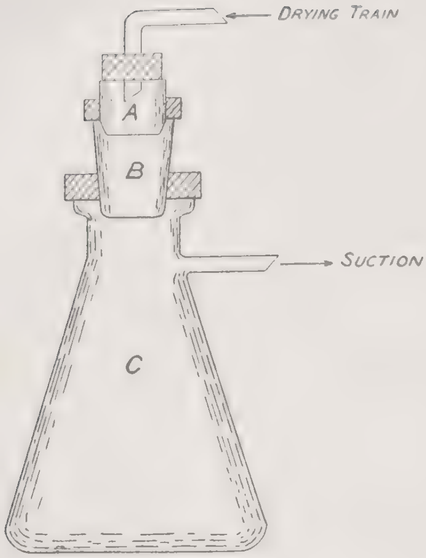

FIG. 36.—APPARATUS FOR DRYING PENTABROMACETONE BY ASPIRATION

gooch, 35 mm diam., for pentabromacetone; and C is 500 ml suction flask. Dry air by passing thru H_2SO_4 and soda-lime, and finally filter thru cotton. Cool air entering drying train by passing thru spiral condenser cooled with H_2O.

Let crucible, B, contg the pentabromacetone, remain under suction ca 1 min. to remove surface moisture before placing in app. If air does not pass thru freely, place crucible in desiccator short time. Maintain slow uniform flow of air by just "cracking" suction.

Total Malic Acid (Laevo- and Inactive) (*28*)—First Action

(Either iso-citric acid or tartaric acid or both may be present)

20.050 REAGENTS

(**a**) *Solvent.*—Either 30% *tert*-amyl alcohol, or 30% *n*-butyl alcohol in $CHCl_3$. (Eastman's Practical *tert*-amyl alcohol and USP $CHCl_3$ without further treatment have been found satisfactory.)

(**b**) *Silicic acid suitable for chromatography.*—*See* **19.015**(**a**).

20.051 APPARATUS

(**a**) *Chromatographic tubes.*—Approx. 13 mm i. d. and 400 mm long; may have perforated disk or coarse fritted disk at beginning of constriction. Glass piston to fit tube.

(**b**) *Source of pressure.*—*See* **18.015**(**c**).

20.052 STANDARDIZATION OF SILICIC ACID COLUMN

Mix in mortar, to uniform powder, 6 g silicic acid and amount of $0.5N$ H_2SO_4 that will allow solvent, **20.050**(**a**), to elute at rate of 1–1.5 ml/min. with pressure of <1 atmosphere. Amount of $0.5N$ H_2SO_4 required may vary with different batches of silicic acid; however, silicic acid and $0.5N$ H_2SO_4 must be measured accurately and column for detn made up exactly as under stdzn. (3 ml $0.5N$ H_2SO_4 has been found satisfactory for 1 batch of silicic acid.) Slurry with enough $CHCl_3$ to fill tube. Place small amount of cotton at bottom of tube and pour slurry into tube so that no air bubbles are occluded. Cut disk of filter paper with cork borer to fit tightly inside tube and pack the silicic acid with piston until no more $CHCl_3$ is forced out. Column packed in this manner permits sample to be stirred with solvent without disturbing column and gives sharper sepn of acids. Remove piston, pour remaining $CHCl_3$ out top of tube, and place 10 ml cylinder under tube.

Dissolve ca 10 mg malic acid in 1 ml $5N$ H_2SO_4 in small beaker. Stir with 2 g silicic acid, or enough to make free-flowing powder that does not adhere to beaker. Transfer thru funnel to column, rinse beaker with ca 5 ml of the solvent, and pour thru funnel into tube. With long thin rod stir powder and solvent in tube until all air bubbles are removed. Remove rod and stand it in sample beaker.

With pressure, pack sample until solvent just disappears into gel. Rinse the long rod, beaker, and funnel with ca 2 ml solvent and sink into gel. Repeat washing with another 2 ml solvent. Place plug of cotton in top of tube, wet it with solvent, and with rod push it down to top of sample. Fill tube with solvent and apply pressure so that eluate is forced out at rate of 1–1.5 ml/min. Titr. eluate in 10 ml portions, rinsing cylinder with 10 ml CO_2-free H_2O and using thymol blue indicator, **35.095**(**k**). If mixt. being titrd is swirled gently so that no emulsion is formed, end point is sharp and easily seen. When excess acid is present, indicator goes into lower layer and as neutrality is reached, indicator turns yellow and enters aq. phase. Swirl and add alkali until lower layer is colorless and aq. layer is blue. Note vol. of solvent required to bring the malic acid to bottom of gel (threshold vol.) and vol. required to elute all the malic acid.

In same manner, det. threshold vol. for citric acid. Vol. solvent necessary to bring citric acid to bottom of column should be at least 20 ml more than that required to elute all the malic acid. During elution, column will become semi-transparent at top and progressively downward, but when malic acid is all eluted, 1 cm or more of column should be unchanged in appearance. When semi-transparency reaches bottom of column, H_2SO_4 may be carried into eluate. (It has been found that first 70 ml eluate contains no malic acid and that next 70 ml contains all the

malic acid and no citric acid. However, vol. required should be detd for app. and particular batch of silicic acid used.)

20.053 DETERMINATION

Proceed as in tartaric acid method, **20.045**, thru par. 2, sentence 3. (The 30% KOH and device for filtering at 0° are not used.) Conc. 200 ml filtrate (do *not* neutralize) to ca 15 ml. Transfer with small amount of H_2O to tared beaker with bottom ca 3 cm diam. and evap. on steam bath to 1 ± 0.5 g. Jet of air over surface of liquid may be used to hasten evapn but no portion of bottom of beaker should be allowed to dry, as darkening of soln may occur with loss of malic acid.

Cool, add 0.25 ml H_2SO_4 (1+1) and 2 g silicic acid or slightly more if necessary to make free flowing powder, and transfer to column prepd as above. Discard vol. eluate equal to detd threshold vol. of malic acid and collect in 150 ml beaker the eluate that will contain all the malic acid and no citric. Evap. solvent on steam bath. (Jet of air over liquid hastens evapn and reduces danger of loss by bumping.) Dissolve residue in ca 10 ml CO_2-free H_2O and titr. with $0.02N$ NaOH, using phthln. Correct titrn for blank on eluate put thru column as above. 1 ml $0.02N$ NaOH = 1.34 mg malic acid; mg malic acid/0.64 = total malic acid in sample.

Acidify soln contg the neutralized malic acid with drop $1N$ HOAc, evap. to ca 15 ml, transfer to 25 ml vol. flask, and proceed as in **20.061**, "and dil. to mark with H_2O." Total malic acid in sample minus laevo-malic acid = inactive malic acid.

Citric and Isocitric Acids

Chromatographic Method (29)—First Action

20.054 REAGENTS

(**a**) *tert-Amyl alcohol in chloroform, 30%.*—Wash USP $CHCl_3$ 3 times with ca 0.5 vol. H_2O to remove alcohol. Dil. 300 ml *tert*-amyl or *n*-butyl alcohol to 1 L with the washed $CHCl_3$ and shake well with ca 50 ml H_2O. Let liquids sep. and discard H_2O. To *tert*-amyl alcohol-$CHCl_3$ layer add excess of anhyd. powd. Na_2SO_4. Shake well and filter thru dry paper.

(**b**) *tert-Amyl alcohol in chloroform, 40%.*—Prep. as in (**a**), using 400 ml *tert*-amyl or *n*-butyl alcohol.

(**c**) *Silicic acid suitable for chromatography.*—*See* **19.015(a)**.

(**d**) *Lead acetate soln.*—Dissolve 75 g normal $Pb(OAc)_2.3H_2O$ in H_2O, add 1 ml HOAc, and dil. to 250 ml.

(**e**) *Metaphosphoric acid.*—20%. Store in refrigerator.

(**f**) *Sodium sulfide soln.*—Dissolve 4 g $Na_2S.9H_2O$ in H_2O and dil. to 100 ml. Store in refrigerator.

(**g**) *Sodium thiosulfate std soln.*—$0.01N$ (2.482 g/L). Stdze against $0.01N$ KIO_3 (0.3567 g/L) as follows: To 5 ml of the KIO_3 soln add 1 ml $2M$ H_3PO_4 and 1 ml 10% KI, and titr. with the $Na_2S_2O_3$ soln, using starch indicator at end point.

(**h**) *Filter paper.*—Cl-free, 9 cm. Wash well with hot H_2O and dry.

(**i**) *Sodium hydroxide std soln.*—$0.01N$. Protect from CO_2.

(**j**) *Potassium chloride soln.*—0.9319 g dried KCl/L of $0.085M$ H_3PO_4.

(**k**) *Silver iodate.*—Protect from light.

20.055 APPARATUS

(**a**) *Chromatographic tube.*—Approx. 13 mm i.d. and 400 mm long; piston to fit tube for packing silicic acid. Plug end of tube with cotton.

(**b**) *Centrifuge tube.*—Approx. 3×11 cm; 60 ml capacity.

(**c**) *Device for titrating in CO_2-free atmosphere.*—125 ml pear-shaped separator with rubber stopper having 5 holes for following: (1) tube, with drawn-out tip extending to stopcock for CO_2-free air; (2) std acid buret tip; (3) std alkali buret tip; (4) funnel for transferring eluate; and (5) tube for exhaust vapors.

(**d**) *CO_2-free air.*—Pass air (conveniently obtained from pressure app., **18.015(c)**) twice thru 20% NaOH soln and then thru H_2O contg phthln and enough $0.1N$ NaOH to produce pink color.

20.056 STANDARDIZATION OF SILICIC ACID COLUMN

Mix thoroly, in mortar, 6 g silicic acid and amount of $0.5N$ H_2SO_4 detd as follows: Ignite ca 1 g silicic acid, accurately weighed, in small crucible at red heat ca 15 min. (gas burner is satisfactory). Cool in efficient desiccator and weigh. Calc. ml $0.5N$ H_2SO_4 required, V, in formula: $V = W(1.9A - 1)$; where W = g silicic acid used for column and A = ratio anhyd. to hydrous silicic acid.

Add $CHCl_3$, little at time, and mix, making uniform slurry that pours readily. With $CHCl_3$ wash bottle, transfer *all* of slurry to chromatographic tube, pouring it down thin rod, and stirring until all air bubbles are removed. Cut circle of coarse filter paper (Whatman No. 4 or equiv.) with cork borer to fit snugly in tube. Sat. with $CHCl_3$ and push down with piston until silicic acid is packed in firm column. Remove piston, letting paper remain at top of column. Just before transferring sample to column, pour off excess $CHCl_3$ and place empty graduate under tube.

Prep. 5 ml of aq. soln of citric and isocitric acids, contg total acidity of ca 12 ml $0.01N$ (ca 4

mg each acid). (If laevo and inactive malic acids and tartaric acid are also included, total acidity should be ca 30 ml $0.01N$—ca 4 mg each acid.) Transfer the 5 ml acid soln to centrifuge tube, (**b**), and add $1N$ NaOH until alk. to phthln plus 2 drops excess. Heat in boiling H_2O 15 min., cool to ca 20°, and add 5.5 vols alcohol, 0.5 ml $1N$ HOAc, and 0.5 ml $Pb(OAc)_2$ soln, (**d**). Mix at intervals or continuously 5 min., centrifuge, and decant clear supernatant. Test liquid with drop of $Pb(OAc)_2$ soln, and if ppt forms in 1 min., add it to ppt in centrifuge tube. Stir or mix ppt with ca 20 ml acetone, centrifuge, and decant and discard acetone. Lay tube on side until acetone evaps or remove it with very gentle current of air at room temp. When ppt is dry, add 0.5 ml $2N$ H_2SO_4 and mix with rod to smooth slurry. Add 1 g silicic acid and mix until powder does not adhere to sides of tube, adding little more silicic acid if necessary.

Transfer thru funnel to prepd column, rinse centrifuge tube with ca 5 ml 30% *tert*-amyl alcohol in $CHCl_3$, (**a**), and pour thru funnel. With long, thin rod stir powder and solvent until all air bubbles are removed. Apply pressure, **18.015**(**c**), to column until solvent just sinks into gel. Wipe centrifuge tube, funnel, and rod with cotton, and place cotton in chromatographic tube; rinse centrifuge tube, funnel, and rod with 2 ml solvent, pour into cotton, and push cotton to top of gel. Let solvent sink into gel. Add ca 200 ml solvent to reservoir and apply pressure until solvent elutes at rate of 1–1.5 ml/min.

Transfer eluate in 10 ml portions promptly (*see* NOTE) to titrg app., (**c**). Rinse graduate with 10 ml freshly washed neutral $CHCl_3$ and then with 10 ml CO_2-free H_2O. Add thymol blue indicator, **35.095**(**k**), and $0.01N$ NaOH until, after thoro mixing by forcing CO_2-free air thru app., lower layer is colorless and upper aq. layer is blue. Back-titr. with std acid and alkali until 1 drop of alkali produces blue color of indicator.

From titrn values det. threshold vol. and vol. required to elute each acid for particular app. and reagents used. Acids elute in following order: Unremoved HOAc is eluted in second and third 10 ml; both inactive and laevo-malic acids appear in 100–160 ml fractions. When malic acid is all removed (ca 170 ml), pour off remaining solvent, add 40% *tert*-amyl alcohol in $CHCl_3$, and continue elution. Both citric and isocitric acids appear in 180–300 ml fractions. Continue elution until tartaric acid is eluted (ca 330–440 ml). Titr. eluate contg citric and isocitric acids immediately.

20.057 DETERMINATION

Take quantity of sample prepd as in **20.002** with titratable acidity ca 30 ml $0.01N$ and with solids content not >2 g. Transfer to centrifuge tube, **20.055**(**b**), adjust vol. to 5 ml by evapn or addn of H_2O, and proceed as in stdzn, beginning "... add $1N$ NaOH until alk. ..." and continue to "Back-titr. with std acid and alkali ..."

(**a**) *Total citric and isocitric acids.*—After malic acid is eluted, change to 40% *tert*-amyl alcohol in $CHCl_3$, elute, and titr. 10 ml aliquots promptly as in **20.056.** 1 ml $0.01N$ NaOH = 0.64 mg anhyd. citric and isocitric acids. Correct titrn for blank. After each titrn collect lower layer and aq. layer in sep. containers. After citric and isocitric acids are eluted, wash combined lower layers with small amount of H_2O and alkali, sep., and add aq. portion to titrd combined citric and isocitric acid solns. Save this soln for detn of normal citric acid.

NOTE: If eluted acid is allowed to stay in contact with eluate, some esters may be formed, causing low results. After titrn, aq. solns may be held until convenient to det. normal citric acid.

(**b**) *Isocitric acid.*—Subtract normal citric acid from total citric acid to obtain isocitric acid.

(**c**) *Determination of reagent blank.*—Prep. silicic acid column as **20.056,** add to it 1 g silicic acid and 0.5 ml $2N$ H_2SO_4, elute, and titr. as in **20.056.**

(**d**) *Normal citric acid* (*30*).—Adjust soln contg the citric and isocitric acids to convenient vol. (50 ml or less) and take aliquot contg not >4 mg citric acid as estimated from titrn of the fraction. Add 2 ml H_2SO_4 to aliquot, cool, and hold below 22°. Add 1 ml 20% metaphosphoric acid, dil. to ca 35 ml, add 2 ml 12% KBr and 5 ml 4% $KMnO_4$, mix, and hold 10 min., without stirring, below 22°. Cool to ca 10° and add cold 3% H_2O_2 dropwise, while stirring, until soln is colorless.

Transfer to 125 ml separator and rinse container with ca 25 ml petr. ether, adding rinsings to separator. Shake well, sep., and discard aq. portion. Wash petr. ether 4 times with ca 3 ml portions H_2O, draining and discarding aq. layer each time. Halides must be completely removed from petr. ether and tip of funnel. Add 3 ml of the Na_2S soln to the petr. ether, shake well, and drain aq. layer into 25 ml vol. flask. Ext. with another 3 ml Na_2S soln and wash with 2 ml portions H_2O until all color is removed, draining both exts and washings into flask. Discard petr. ether. Add 2.0 ml $2M$ H_3PO_4 to contents of flask, mix, and then add very small quartz or porcelain chip to facilitate smooth boiling and boil 5–6 min. Cool, and add *exactly* 5.00 ml of the KCl soln, **20.054**(**j**). Dil. to mark and transfer soln, without rinsing, to 50 ml erlenmeyer contg 0.25 g dry $AgIO_3$, **20.054**(**k**). Shake vigorously 5 min. and filter immediately thru dry Cl-free paper.

To 5 ml filtrate add 1 ml 10% KI soln and 2 drops *0.085M* H_3PO_4, and titr. at once with $0.01N$

$Na_2S_2O_3$, using starch indicator. Correct titrn for blank detn on 5 ml H_2O and 6 ml Na_2S soln in 25 ml vol. flask, beginning as above, "Add 2.0 ml 2*M* H_3PO_4 . . ." Blank titrn includes value for KCl as well as any halide in reagent. Ml 0.01*N* $Na_2S_2O_3 \times 0.064$ = mg anhyd. normal citric acid in filtrate aliquot.

Laevo-Malic Acid

Method I. (*31*)—*First Action*

(As method is empirical, all directions must be rigidly followed, particularly with respect to dilns. Substitution of vol. flasks of capacities different from those specified is not permissible.)

20.058 REAGENTS

(**a**) *Lead acetate soln.*—Dissolve 40 g $Pb(OAc)_2$ in H_2O, add 0.5 ml HOAc, and dil. to 100 ml.

(**b**) *Tribasic lead acetate std soln.*—Prep. soln from tribasic $Pb(OAc)_2$, (**c**). To 5 g of the salt in 500 ml erlenmeyer add 200 ml H_2O and shake vigorously. Neutralize 3 ml 1*N* H_2SO_4, dild with 200 ml H_2O, with the soln, using Me red as indicator. Note vol. Pb soln required. In detn use 2 ml in excess of this quantity. (Soln should be freshly prepd.)

(**c**) *Tribasic lead acetate.*—Dissolve 82 g $Pb(OAc)_2$ in 170 ml H_2O. Prep. 100 ml dil. NH_4OH soln contg 5.8 g NH_3 as detd by titrn (Me red). Heat solns to 60°, mix thoroly, and let stand overnight. Shake vigorously to break up ppt, and filter on büchner. Wash once with H_2O and suck dry, then twice with alcohol, and finally with ether. Let dry in air.

20.059 PREPARATION OF SAMPLE

Proceed as in **20.044,** omitting addn of the 3 ml 1*N* H_2SO_4 to adjusted sample. In case of saponification add *A* +3 ml 1*N* H_2SO_4 to saponified material instead of *A* +6 ml.

20.060 DETERMINATION

(**a**) *Isolation of laevo-malic acid.*—To material in centrifuge bottle add ca 75 mg *tartaric acid* and quantity of the $Pb(OAc)_2$ soln, **20.058**(**a**), equal to *A* (*A* +3 ml in case saponification was made), shake vigorously 2 min., and centrifuge. Carefully decant supernatant from pptd Pb salts and test with small quantity of the $Pb(OAc)_2$ soln. If ppt forms, return to centrifuge bottle, add more $Pb(OAc)_2$ soln, shake, and again centrifuge. If sediment lifts, repeat centrifuging, increasing speed and time. Let ppt drain thoroly by inverting bottle several min.

Add ca 200 ml 80% alcohol, shake vigorously, add again centrifuge, decant, and drain. To Pb salts add ca 150 ml H_2O, shake vigorously, and pass in rapid stream of *H_2S to saturation.* Stopper bottle and shake ca 1 min. Transfer mixt. to 250 ml vol. flask with H_2O, dil. to mark, shake, and filter thru folded paper.

Pipet 220 ml filtrate into 600 ml beaker and evap. on gauze to ca 50 ml. Cool, neutralize with 1*N* KOH (phthln), and add 5 drops excess. Add 2 ml HOAc and transfer with alcohol to 250 ml vol. flask. Add alcohol to mark, shake, and pour into 500 ml erlenmeyer. Add small handful of glass beads and cool to 15°. Stopper flask, shake vigorously 10 min., and place in refrigerator 30 min. Again shake 10 min., and filter thru folded paper.

Pipet 220 ml clear filtrate into centrifuge bottle, add $Pb(OAc)_2$ soln equal to *A* (*A* +3 ml in case of saponification), shake vigorously ca 2 min., centrifuge, decant, and drain. Add 200 ml 80% alcohol, shake, centrifuge, decant, and drain.

Transfer Pb salts to 500 ml erlenmeyer with ca 175 ml H_2O. Add 3 ml 1*N* H_2SO_4, heat to boiling, and add 1 ml HOAc (5+95) and quantity of the std tribasic $Pb(OAc)_2$ soln previously detd, **20.058**(**b**). Boil mixt. 5 min., cool to room temp., transfer to 250 ml vol. flask with H_2O, dil. to mark, shake, and pour into 500 ml erlenmeyer. Add small handful of glass beads, cool to 15°, shake vigorously 5 min., and place in refrigerator 30 min. Again shake 5 min. and filter thru folded paper. Sat. *clear* filtrate with H_2S, shake vigorously, and filter.

(**b**) *Polarization.*—Evap. 225 ml of the clear filtrate over gauze to ca 10 ml, neutralize with 1*N* KOH (phthln), make slightly acid with HOAc (5+95), and evap. to ca 5 ml. Transfer to 25–27.5 ml Giles flask with H_2O, dil. to 27.5 ml mark, shake, and pour into small g-s. erlenmeyer. If Giles flask is not available, use 25 ml measuring cylinder, dil. to mark, and add 2.5 ml H_2O from buret. Add small handful of glass beads and 4 g powd. *uranyl acetate*, shake vigorously 10 min., and filter. (As U-malic complex is light sensitive, wrap flask in towel while shaking and protect from light as much as possible during filtration and polarization.) Polarize in 200 mm tube at 20°, using white light. After filling tube, release tension on glass disks by slightly loosening caps, and let stand at 20° at least 30 min. before making readings.

°S (**29.020**(**a**)) $\times 30.1$ = mg laevo-malic acid contained in portion taken for analysis. If control for adjusting to std temp. 20° is lacking, det. temp. of polariscope and at this temp. prep. soln of the U-complex as above. Make readings after letting tube remain in trough of instrument 30 min.

20.061 *Method II.* (*32*)—*First Action*

(Not applicable in presence of iso-citric acid blackberry)

Conc. filtrate from tartaric acid detn, **20.045**, to ca 5 ml on steam bath. (Jet of air over surface of

liquids speeds evapn and reduces danger of loss by bumping.) Cool, add NaOH (1+1) drop at time until alk. to phthln, and then add just enough 1*N* HOAc to discharge phthln color. Transfer to 25 ml vol. flask and dil. to mark with H_2O.

Pour soln into fine porosity fritted filter tube contg mat of C several mm thick. (Merck's activated charcoal for decolorizing and Nuchar W have been found satisfactory.) Force liquid slowly thru disk, 1–2 ml/min., into 50 ml flask, using pressure. If soln is not colorless, pass thru another fresh C mat. Mix soln and polarize in 200 mm tube at room temp., using white light. Return soln in polariscope tube to remainder in flask. Add 2.5 g finely powd. *uranyl acetate, protect from light,* and shake in machine 0.5 hr.

Filter on retentive paper in *dark*, mix, and polarize as before. Do not let treated soln be exposed to light, which causes the U-complex to become insol., and if it is filtered off, loss of malic acid occurs. Algebraic difference between readings in °S (**29.020**(a)) ×factor 15.3 gives mg laevo-malic acid in sample taken for tartaric acid detn.

Inactive Malic Acid (*33*)—First Action

(As method is empirical, all directions must be rigidly followed, particularly with respect to dilns. Substitution of vol. flasks of capacities different from those specified is not permissible.)

20.062 REAGENTS

Use reagents described in **20.058** and in addn—

(a) *Potassium permanganate soln.*—Dissolve 14.5214 g purest $KMnO_4$ in H_2O and dil. to 1 L. Stdze soln as follows: Pipet 50 ml oxalic acid soln, (b), into 600 ml beaker and add 70 ml H_2O and 10 ml H_2SO_4 (1+1). Heat to 80°, immediately add the $KMnO_4$ soln to faint pink, again heat to 80°, and finish titrn. 50 ml of the $KMnO_4$ soln should be equiv. to 50 ml of the oxalic acid soln.

(b) *Oxalic acid soln.*—Dissolve 28.7556 g purest $H_2C_2O_4.2H_2O$ in H_2O and dil. to 1 L. (1 ml = 5 mg malic acid (laevo or inactive)).

20.063 PREPARATION OF SAMPLE

Subject 2 portions of sample to isolation procedure, **20.064**(a); use one portion for detn of laevo-malic acid (polarization), **20.064**(b), and other for total malic acid, laevo+inactive (oxidation), **20.064**(c). Choose quantity of sample with titratable acidity not >150 mg acid calcd as malic acid. Designate as *A* ml 1*N* alkali required to neutralize quantity of sample chosen. In no case should solids content be >20 g (200 ml sample soln of jam or jelly).

Adjust sample soln to ca 35 ml by evapn or addn of H_2O, pour into 250 ml vol. flask, rinse with 10 ml hot H_2O and then with alcohol, and dil. to mark with alcohol. Shake, let stand until pectin seps, leaving clear liquid, overnight if necessary, and filter thru folded paper, draining thoroly and covering funnel with watch glass. Pipet 225 ml filtrate into centrifuge bottle.

20.064 DETERMINATION

(a) *Isolation of total malic acid.*—To soln in centrifuge bottle add ca 25 mg *citric acid* and quantity $Pb(OAc)_2$ soln, **20.058**(a), equal to *A* (*A*+3 ml if saponification was made), shake vigorously 2 min., and centrifuge. Carefully decant supernatant from pptd Pb salts and test with small quantity of the $Pb(OAc)_2$ soln. If ppt forms, return to centrifuge bottle, add more $Pb(OAc)_2$, shake, and again centrifuge. If sediment lifts, repeat centrifuging, increasing speed and time. Let ppt drain thoroly by inverting bottle several min.

Add 200 ml 80% alcohol, shake vigorously, and again centrifuge, decant, and drain. To Pb salts add ca 150 ml H_2O, shake vigorously, and pass in rapid stream of *H_2S to saturation.* Stopper bottle and shake ca 1 min. Transfer mixt. to 250 ml vol. flask with H_2O, dil. to mark, shake, and filter thru folded paper.

Pipet 225 ml filtrate into 600 ml beaker, and evap. to ca 100 ml to expel H_2S. Transfer to 250 ml vol. flask with H_2O. (Vol. in flask should be ca 200 ml.) Add 5 ml HOAc (1+9) and same quantity of $Pb(OAc)_2$ soln previously used. Shake vigorously, dil. to mark with H_2O, and filter.

Pass rapid stream of H_2S into *clear* filtrate *to saturation*, stopper flask, shake vigorously, and filter. Pipet 225 ml filtrate into 600 ml beaker, add ca 75 mg *tartaric acid*, and evap. on gauze to ca 50 ml. Cool, neutralize with 1*N potassium hydroxide* (phthln), and add 5 drops excess. Add 2 ml HOAc and transfer mixt. to 250 ml vol. flask with alcohol. Dil. to mark with alcohol, shake, and pour into 500 ml erlenmeyer. Add small handful of glass beads and cool to 15°. Stopper flask, shake vigorously 10 min., and place in refrigerator 30 min. Again shake 10 min. and filter thru folded paper.

Adjust *clear* filtrate to 20° and pipet 225 ml into centrifuge bottle. Add $Pb(OAc)_2$ soln equal to *A* (*A*+3 ml if saponification was made), shake vigorously ca 2 min., centrifuge, decant, and drain. Add 200 ml 80% alcohol, shake, centrifuge, decant, and drain.

Transfer Pb salts to 500 ml erlenmeyer with ca 175 ml H_2O. Add 3 ml 1*N* H_2SO_4 and heat to boiling; add 1 ml HOAc (5+95) and quantity std tribasic $Pb(OAc)_2$ soln previously detd in **20.058**(b). Boil mixt. 5 min., cool to room temp., transfer to 250 ml vol. flask with H_2O, dil. to mark, shake, and pour into 500 ml erlenmeyer. Add small handful of glass beads, cool to ca 15°, shake vigorously 5 min., and place in refrigerator

30 min. Again shake 5 min. and filter thru folded paper. Sat. *clear* filtrate with H_2S, shake vigorously, and filter. Use one of the two portions for polarization and other for oxidation.

(**b**) *Polarization.*—Evap. 225 ml clear soln over gauze to ca 10 ml and proceed as in **20.060(b)**. °S (**29.020**(a)) $\times 10.2 =$ mg laevo-malic acid contained in aliquot (l in formula (**d**)).

(**c**) *Oxidation.*—Evap. 225 ml clear soln to ca 10 ml to expel last traces of alcohol, dil. to ca 120 ml with H_2O, and add 10 ml 30% NaOH soln and 25 ml of the $KMnO_4$ soln. Heat to ca 80° and keep in boiling H_2O bath 30 min. Add 25 ml of the oxalic acid soln and 10 ml H_2SO_4 (1+1), stirring vigorously. Adjust to 80°, and titr. to faint pink with the $KMnO_4$ soln. Again heat to 80° and finish titrn. Ml $KMnO_4$ soln used $\times 5 =$ total oxidizable material (as malic acid) present in aliquot (t in formula (**d**)).

(**d**) *Calculation.*—Calc. mg inactive malic acid, X, in portion taken for analysis by following formula: $X = 4(t-5-l)$, where $t =$ mg oxidizable as malic acid; $l =$ mg laevo-malic acid; $5 =$ correction factor for mg non-malic material as malic acid; and $4 =$ factor for reverting inactive malic acid in aliquot back to quantity of inactive acid in sample taken for analysis.

20.065 Lactic Acid (*34*)—Official

Pipet 200 ml prepd soln, **20.002(c)**, into 400 ml beaker and evap. to 50 ml. Cool and transfer contents of beaker to 250 ml vol. flask with alcohol. Dil. to mark with alcohol, shake, and filter thru folded paper. Pipet 200 ml filtrate into 400 ml beaker and evap. to ca 25 ml. Add 50 ml H_2O and again evap. to 25 ml. Transfer material to continuous extractor, Fig. 26, page 187, with 25 ml H_2O and proceed as in **15.012–15.013.**

Sucrose—Official

20.066 *By Polarization*

Det. by polarizing before and after inversion. *See* **29.025, 29.026,** or **29.031.**

20.067 *By Reducing Sugars Before and After Inversion*

Transfer sample representing (if possible) ca 2.5 g total sugars to 200 ml vol. flask; dil. to ca 100 ml and add excess of satd neutral $Pb(OAc)_2$ soln, **29.021(d)** (ca 2 ml is usually enough). Mix, adjust to 200 ml, and filter, discarding first few ml filtrate. Add dry K or Na oxalate to ppt excess Pb used in clarification, mix, and filter, discarding first few ml filtrate. Take 25 ml filtrate or aliquot contg (if possible) 50–200 mg reducing sugars and proceed as in **29.039 29.040.**

For inversion at room temp. transfer 50 ml aliquot clarified and deleaded soln to 100 ml vol. flask, add 10 ml HCl (1+1), and let stand at room temp. (20° or above) 24 hr; exactly neutralize with concd NaOH soln, using phthln, and dil. to 100 ml. Take aliquot and det. total sugars as invert as in **29.039–29.040.**

20.068 Reducing Sugars—Official—*See* 29.039

Express results as invert sugar.

29.069 Commercial Glucose—Procedure —*See* 29.034

Starch

20.070 *Qualitative Test—Official*

Dil. portion of sample with H_2O, heat nearly to boiling, add several ml H_2SO_4 (1+9), and then 10% $KMnO_4$ soln until all color is destroyed. Cool, and test with I soln, **28.025(d)**. (Presence of starch is not necessarily indication of its addn as adulterant. It is usually present in small quantity in the apple, and occasionally in other fruits, and unless it is found in the fruit product in considerable quantity its presence may be due to these natural sources.)

Essential Oil (*35*)—First Action

20.071 APPARATUS

Use app. of **19.102,** substituting 2 L flask with ₸ 24/40 joint.

20.072 DETERMINATION

Place 1 L sample in boiling flask and add few glass beads to facilitate boiling. Fill oil separatory trap with H_2O, connect with boiling flask and condenser, and boil 1 hr. Remove heat and let stand several min. Drain enough H_2O to bring oily layer within graduated portion of trap, let stand at least 5 min. to complete drainage, and measure quantity of oil from bottom of lower meniscus to highest point of upper meniscus.

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(*8*) Ibid. **6,** 34(1922); **21,** 504(1938); **30,** 260 (1947); **32,** 177(1949); **33,** 349(1950).
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(*29*) Ibid. **40,** 333(1957).
(*30*) Anal. Chem. **23,** 467(1951).
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(*33*) Ibid. **16,** 281(1933).
(*34*) Ibid. **20,** 605(1937); **26,** 199(1943).
(*35*) Ibid. **27,** 201(1944).

21. Gelatin, Dessert Preparations, and Mixes

GELATIN

21.001 Preparation of Sample—Procedure

Mix ground gelatin thoroly. Break sheet gelatin into small pieces by hand. Further comminution is unnecessary in either case.

21.002 Moisture—Official

Proceed as in **13.003,** using 2 g sample prepd as in **21.001.**

21.003 Ash—Official—*See* 29.012 or 29.013

21.004 Total Phosphorus—Official

Treat ash, **21.003,** with 2–3 ml HNO_3 and evap. to dryness on steam bath. Repeat HNO_3 treatment and evapn, take up residue in hot H_2O contg few drops HNO_3, and proceed as in **2.019.**

21.005 Nitrogen—Official

Proceed as in **2.036,** using 1 g sample. To convert to ash-free, anhyd. gelatin multiply by factor 5.55 (*1*).

21.006 Jelly Strength (*2*)—Official

Pipet 105 ml H_2O at 10–15° into std Bloom bottle, add 7.5 g sample, and stir. Let stand 1 hr and then bring to 62° in 15 min. by placing in H_2O bath regulated at 65° (sample may be swirled several times to aid soln). Finally mix by inversion, let stand 15 min., and place in H_2O bath at $10 \pm 0.1°$. Chill 17 hr. Det. jelly strength in Bloom Gelometer (*3*), adjusted for 4 mm depression and to deliver 200 ± 5 g shot/5 sec., using the 0.5″ plunger, Fig. 37.

GELATIN DESSERT POWDERS

21.007 Preparation of Sample—Procedure

Sift sample thru No. 30 sieve onto large sheet of paper, rubbing material thru sieve and tapping vigorously, if necessary. Sift sample 2 more times, mixing thoroly each time. To avoid absorption of moisture, operate as rapidly as possible, and store sample in air-tight container.

21.008 Moisture—Official

Proceed as in **29.006,** using 2 g sample prepd as in **21.007.**

21.009 Ash—Official—*See* 29.012 or 29.013

21.010 Nitrogen—Official—*See* 21.005

21.011 Total Acidity—Official

Dissolve 20 g sample in 2 L recently boiled H_2O. Titr. 100 ml with $0.1N$ NaOH, using 0.3 ml phthln. Report as % by wt citric acid.

21.012 Jelly Strength—Official

To 20 g sample in std Bloom bottle, add from pipet, with stirring, 100 ml H_2O at 10–15°. Let stand 15 min. and then bring to 62° in 15 min. in H_2O bath regulated at 65° (sample may be swirled several times to aid soln). Mix by inversion, let stand 15 min., place in H_2O bath controlled at $10 \pm 0.1°$, and let stand 17 hr. Det. jelly strength in Bloom Gelometer (*3*), adjusted for 4 mm depression and to deliver 200 ± 5 g shot/5 sec., using the 1.0″ plunger, Fig. 38, and light wt shot receiver (paper or plastic).

Sucrose (*4*)—First Action

21.013 REAGENTS

(**a**) *Tannin soln.*—Dissolve 5 g tannin in 100 ml cold H_2O.

(**b**) *Lead acetate soln.*—Dissolve 100 g $Pb(OAc)_2.3H_2O$ in 200 ml H_2O. (This makes 30° Bé. soln.)

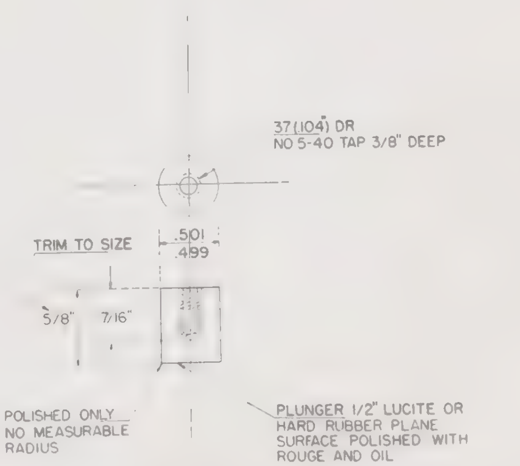

FIG. 37.—0.5 INCH BLOOM GELOMETER PLUNGER

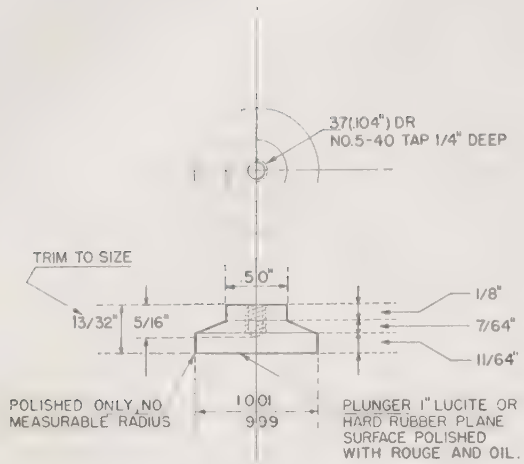

FIG. 38.—1.0 INCH BLOOM GELOMETER PLUNGER

21.014 DETERMINATION

Place 13 g sample in 300 or 400 ml beaker, add 2 g $CaCO_3$ and 2 g Filter-Cel, and mix well with glass rod. Add 175 ml boiling H_2O, creaming mixt. with little of the H_2O at first. Stir thoroly and let stand few min. to insure soln. Cool under cold H_2O to 30°, add slowly with stirring 25 ml of the tannin soln, and let stand 5 min. (This quantity of tannin soln is enough for most powders; if 30 ml is required, use 170 ml H_2O instead of 175 ml.) Add slowly with stirring 10 ml $Pb(OAc)_2$ soln and filter on 18.5 cm Whatman No. 2 paper. (Total quantity of liquid used in each case is 210 ml which yields 200 ml after evapn and concn. If pptn has been conducted properly, soln will filter readily and filtrate will be clear.) Read optical rotation of this soln in 200 mm tube at 20°.

If sample contains reducing sugar, delead with $K_2C_2O_4$, add Filter-Cel, and filter. Invert by placing 50 ml filtrate in 100 ml vol. flask with 5 ml HCl and letting stand overnight. After inversion, neutralize with concd NaOH soln, using phthln. Discharge color of indicator with 0.1N HCl. Cool to 20°, dil. to vol., and read optical rotation in 200 mm tube. Use following Clerget formula modified for % sucrose in gelatin dessert powders:

$$S = \frac{100(4P - 8I)}{142.66 + 0.0676(m - 13) - t/2},$$

where S = % sucrose; P = direct reading; I = invert reading; t = temp. at which readings are made (20°); and m = g total solids from original sample/100 ml invert soln (3.25 g). Simplified:

$$S = 100(4P - 8I)/132.$$

21.015 Dextrose—First Action

Det. polarization due to dextrose (D) by subtracting % sucrose (S) as found in **21.014** from direct reading of polariscope in circular degrees (P) multiplied by 4: $D = 4P - S$.

Calc. % dextrose (D') from following formula:

$$D' = D \times 66.5/52.5 = 1.267D,$$

where D = polarization due to dextrose; 66.5 = specific rotation of sucrose; and 52.5 = specific rotation of dextrose.

STARCH DESSERT POWDERS

21.016 Preparation of Sample—Procedure—*See* 21.007

21.017 Moisture—Official

Proceed as in **29.005** or **29.006**, using 2 g prepd sample, **21.007**.

21.018 Ash—Official—*See* 29.012 or 29.013

21.019 Nitrogen—Official

Proceed as in **2.036**, using 1 g sample. To convert to protein multiply by factor 6.25.

21.020 Sucrose and Dextrose—First Action—*See* 21.014 and 21.015

21.021 Starch—First Action

(**a**) *By direct acid hydrolysis.—See* **22.043**.
(**b**) *Polarimetric method.—See* **13.037**.

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22. Grain and Stock Feeds

22.001 Sampling (*1*)—Procedure

Use slotted single or double tube, or slotted tube and rod, all with pointed ends.

Take at least 1 lb sample, 2 lb preferred, as follows: Lay bag horizontally and remove core diagonally from end to end. Det. number of cores as follows: From lots of 1–10 bags, sample all bags; from lots of 11 or more, sample 10 bags. Take 1 core from each bag sampled, except that for lots of 1–4 bags take enough diagonal cores from each bag to total at least 5 cores. For bulk feeds draw at least 20 cores from different regions; in sampling small containers (10 lb or less) 1 package is enough. Reduce composite sample to quantity required, preferably by riffling, or by mixing thoroly on clean oil-cloth or paper and quartering. Place sample in air-tight container.

A sample from less than these numbers of bags may be declared an official sample if guarantor agrees. For samples that cannot be representatively taken with probe described, use other sampling means.

22.002 Preparation of Sample—Official

Grind sample to pass sieve with circular openings 1/25″ (1 mm) diam. and mix thoroly. If sample cannot be ground, reduce to as fine condition as possible.

Moisture

22.003 *I. Drying in Vacuo at 95–100° (2)—Official*

Dry quantity of sample representing ca 2 g dry material to constant wt at 95–100° under pressure not >100 mm Hg (ca 5 hr). Use covered Al dish at least 50 mm diam. and not >40 mm deep. Report loss in wt as moisture.

II. By Distillation with Toluene (3)—Official

22.004 APPARATUS

250 ml distg flask of Pyrex or other resistant glass connected by means of "distg tube receiver" to 20″ sealed-in, straight-tube Liebig condenser with delivery tube not >5/16″ in diam. as in Fig. 39. Receiver, dimensions shown, is made by attaching proper side tube to calibrated section of 5 ml Mohr pipet and sealing outlet. Tube is calibrated in ml by distg known quantities H_2O into graduated column, and column of H_2O may be read to hundredths with reasonable accuracy. Clean tube and condenser with $K_2Cr_2O_7$-H_2SO_4 mixt., rinse thoroly with H_2O, then alcohol, and dry in oven to prevent undue quantity of H_2O adhering to inner surfaces during detn.

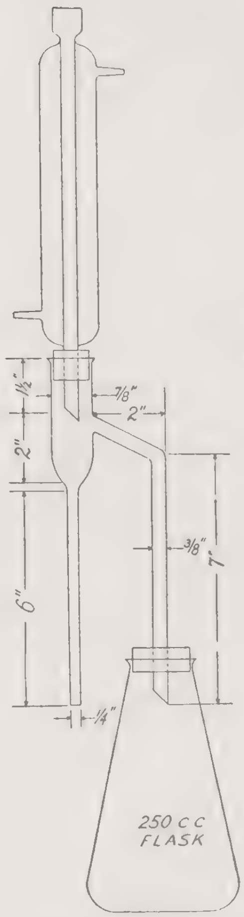

FIG. 39.—APPARATUS USED IN METHOD II FOR DETERMINATION OF MOISTURE

22.005 DETERMINATION

If sample is likely to bump, add dry sand to cover bottom of flask. Add enough toluene to cover sample completely (ca 75 ml). Weigh and introduce into toluene enough sample to give 2–5 ml H_2O and connect app. as shown, Fig. 39. Fill receiving tube with toluene, pouring it thru top of condenser. Bring to boil and distill slowly, ca 2 drops/sec., until most of the H_2O passes over; then increase rate of distn to ca 4 drops/sec.

When all H_2O is apparently over, wash down condenser by pouring toluene in at top, continuing distn short time to see whether any more H_2O will distill over; if it does, repeat washing-down process. If any H_2O remains in condenser, remove by brushing down with tube brush attached to

Cu wire and satd with toluene, washing down condenser at same time. (Entire process is usually completed within 1 hr.) Let receiving tube come to room temp. If any drops adhere to sides of tube, force them down, using Cu wire with end wrapped with rubber band. Read vol. H_2O and calc. to %.

III. Drying without Heat over Sulfuric Acid (4)—Official

22.006 REAGENT

Sulfuric acid.—Boil H_2SO_4 in large Kjeldahl flask 4 hr, close flask with stopper carrying $CaCl_2$ tube, and cool.

22.007 DETERMINATION

Weigh 2–5 g sample into metal dish 5–10 cm diam. with tight-fit cover. (If subsequent fat detns are to be made, fat extn cones may be used.) Mix substances that dry down to horn-like material with fat-free cotton or other suitable material. Place 200 ml of the fresh H_2SO_4 in strong, tight vac. desiccator. Place uncovered dish in desiccator and exhaust with vac. pump to pressure of not >10 mm Hg.

If pump is not available, place 10 ml ether in small beaker in desiccator and exhaust with H_2O filter pump. Between pump and desiccator interpose empty bottle next to desiccator and bottle of H_2O next to pump. Draw air from desiccator thru the H_2O and turn desiccator stopcock the instant H_2O begins to rise in tube leading from empty bottle.

Gently rotate desiccator 4 or 5 times during first 12 hr. After 24 hr open desiccator, causing incoming air to bubble thru H_2SO_4; place cover on dish and make first weighing. After weighing place sample in desiccator contg fresh H_2SO_4 and exhaust as before. Rotate desiccator several times during interval and weigh again after suitable drying period. Repeat process to constant wt.

22.008 *IV. Drying at 135° (5)—Official*

(Not to be used when fat detn is to be made on same sample)

Regulate elec. air oven to 135±2°. Using low, covered Al dishes, **22.003**, weigh ca 2 g sample into each dish and shake until contents are evenly distributed. With covers removed, place dishes and covers in oven as quickly as possible and dry samples 2 hr. Place covers on dishes and transfer to desiccator to cool. Weigh and calc. loss in wt as H_2O.

22.009 *V. In Highly Acid Milk By-products (6)—Official*

Add ca 2 g ZnO, freshly ignited or oven dried, to flat-bottom dish not <5 cm diam. and weigh. Add ca 1 g sample and weigh quickly. Add ca 5 ml H_2O and distribute sample evenly on bottom of dish. Heat on steam bath, exposing max. surface of dish bottom to live steam until apparently dry. Heat at 98–100° in air oven 3 hr or to constant wt. Cool in desiccator; then weigh quickly. Det. wt residue. Titr acidity of sample and calc. as lactic acid, **15.004.** To compensate for H_2O formed when acid is neutralized by ZnO, add 0.1 g to residue wt for each g acid (as lactic) in weighed sample. Report % residue (corrected) as total solids.

22.010 **Ash** (7)—**Official**

Weigh 2 g sample into porcelain crucible and place in muffle furnace preheated to 600°. Hold at this temp. 2 hr with automatic control pyrometer. Transfer crucibles directly to desiccator, cool, and weigh immediately, reporting % ash to first decimal place.

22.011 **Crude Protein** (8)—**Official**

Det. N as in **2.036.** Multiply result by 6.25, or in case of wheat grains by 5.70.

Qualitative Tests for Proteins (9)—**Official**

Biuret Test

(Unreliable in presence of glycerol)

(Biuret test depends on peptide grouping, -HNCONH-, and therefore is given by all proteins. It is also given by certain other compounds contg similar groupings, such as biuret,

$H_2NCONHCONH_2$,

and malonamide,

$H_2NCOCH_2CONH_2$.

Compounds contg one $-CONH_2$ and one $-CSNH_2$, $-C(NH)NH_2$, or $-CH_2NH$ similarly joined also respond to this test.)

22.012 REAGENT

Add slowly, with stirring, 25 ml 3% $CuSO_4.5H_2O$ soln to 1 L 10% NaOH soln. If necessary to filter reagent, use glass wool.

22.013 DETERMINATION

(**a**) To 2 or 3 ml protein soln add, with shaking, few drops of reagent. If characteristic pink or violet color does not develop quickly, let stand 15 or 20 min. In presence of $(NH_4)_2SO_4$ addn of NaOH is necessary.

(**b**) *Osborne modification.*—This modification of biuret test greatly increases its delicacy. Proceed as in (**a**); then add 10–20 drops alcohol and some solid NaOH (ca 5 g). The alkali salts out the small quantity of alcohol, which carries with it the color present, and in this way presence of small quantities of protein can be detected.

Millon Test

(Given by all aromatic substances, such as phenol and salicylic acid, which contain benzene nucleus with substituted hydroxyl group. In pro-

teins this grouping is furnished by amino acid tyrosine.)

22.014 REAGENT

Dissolve, by gently warming, 1 part by wt Hg in 2 parts by wt HNO_3. Dil. with 2 vols H_2O. Let mixt. stand overnight and decant supernatant. Soln contains $Hg(NO_3)_2$, $HgNO_3$, HNO_3, and some HNO_2.

22.015 TEST

Add few drops of reagent to 4 or 5 ml protein soln in test tube. Warm gently by immersing few min. in hot H_2O. Pink or red color develops slowly and ppt usually forms. If sample is solid, suspend in 3 or 4 ml H_2O and treat as above. Alk. solns should first be neutralized to avoid pptn of HgO.

Glyoxylic Acid Test (Hopkins-Cole)

22.016 REAGENT

Add H_2O to liberally cover 10 g powd. Mg in large erlenmeyer. Add 250 ml cold satd oxalic acid soln, cooling flask under H_2O tap during addn. After reaction is over, shake mixt. and filter. Acidify filtrate with HOAc and dil. to 1 L with H_2O.

22.017 TEST

To 1 or 2 ml protein soln in test tube add 3 ml of reagent and mix thoroly. Using pipet, let mixt. flow gently down side of second test tube (slightly inclined) contg 5 ml H_2SO_4. Reddish-violet color forms at junction of fluids, owing to presence of tryptophane in the protein.

22.018 *Adamkiewicz Test*

Proceed as in **22.017,** except to use HOAc instead of prepd soln of glyoxylic acid. Color reaction depends on presence of traces of glyoxylic acid formed from the HOAc.

22.019 *Xanthoproteic Test*

Add ca 1 ml HNO_3 to 3 ml of the protein soln. White ppt forms, which on boiling becomes yellow and may dissolve to give yellow soln. Cool and make slightly alk. by careful addn of 30% NaOH soln. Color changes to deep orange. Color development depends on formation of nitro derivatives attached to benzene nucleus, present in amino acids tyrosine and phenylalanine of proteins.

Albuminoid Nitrogen—Official

22.020 REAGENT

Cupric hydroxide.—Dissolve 100 g $CuSO_4.5H_2O$ in 5 L H_2O; add 2.5 ml glycerol and then 10% NaOH soln until liquid is slightly alk. Filter, rub ppt in mortar with H_2O contg 5 ml glycerol/L, and wash by decanting or filtering until washings are no longer alk. Then rub ppt in mortar with H_2O contg 10% glycerol, thus prepg uniform gelatinous mass that can be measured with pipet. Det. approx. quantity of $Cu(OH)_2$ in 5 ml by dilg to 50 ml with H_2O, filtering, washing, igniting, and weighing as CuO.

22.021 DETERMINATION

Place 0.7 g sample in beaker, add 100 ml H_2O, and heat to boiling; or, in case of substances rich in starch, heat on steam bath 10 min. Add quantity of reagent, **22.020,** contg ca 0.5 g $Cu(OH)_2$, stir thoroly, filter when cold, wash with cold H_2O, and without removing ppt from filter, det. N as in **2.036,** adding enough K_2S or Na_2S soln, **2.034(e)**, to ppt all Cu and Hg. (Filter paper must be practically N-free.) If material (such as seeds, seed residue, or oil cake) is rich in alk. phosphates, add 1–2 ml 10% soln of *NH_3-free $NaAl(SO_4)_2.12H_2O$* to decompose them, then the $Cu(OH)_2$, and mix well by stirring. If this is not done, $Cu_3(PO_4)_2$ and free alkali may be formed, and the protein-Cu ppt may partially dissolve in the alk. liquid.

22.022 **Amido Nitrogen—Official**

% total N − % albuminoid N = % amido N.

Urea and Ammoniacal Nitrogen (*10*)—Official

22.023 REAGENTS

(**a**) *Defoaming soln.*—Dissolve 50 g diglycol stearate in 375 ml benzene, 75 ml alcohol, and 250 ml dibutyl phthalate, warming if necessary.

(**b**) *Urease soln.*—Prep. fresh soln by dissolving stdzd urease in H_2O so that each 10 ml neutralized soln will convert the N of at least 0.1 g pure urea.

Standardization.—To det. alky of commercial urease prepn dissolve 0.1 g in 50 ml H_2O and titr. with 0.1N HCl, using Me red, **2.034(i)**. Add same quantity 0.1N HCl to each 0.1 g urease in prepg the urease soln. To det. enzyme activity, prep. ca 50 ml neutralized 1% soln. Add different quantities of soln to 0.1 g samples pure urea and follow with enzyme digestion and distn as in detn. Calc. activity of urease prepn from quantity of this urease soln that completely converted the urea, in order to permit complete recovery of the N by distn.

(**c**) *Calcium chloride soln.*—Dissolve 25 g $CaCl_2$ in 100 ml H_2O.

22.024 DETERMINATION

Place 2 g sample in Kjeldahl flask with ca 250 ml H_2O. Add 10 ml urease soln, stopper tightly, and let stand 1 hr at room temp. or 20 min. at 40°. Cool to room temp. if necessary. Use more urease soln if feed contains >5% urea (ca 12% protein equiv.). Rinse stopper and neck with few ml H_2O. Add 2 g or more MgO (heavy type), 1 ml $CaCl_2$ soln, and 5 ml defoamer soln, and connect flask with condenser by Kjeldahl connecting bulb

Distill 100 ml into measured quantity of std acid **2.034(j)**, and titr. with std alkali, **2.034(k)**, using Me red, **2.034(i)**.

Pepsin Digestibility of Animal Protein Feeds (*11*) —Official

22.025 PRINCIPLES

Defatted sample is digested 16 hr with warm acid soln of pepsin under constant agitation. Insol. residue is centrifuged, dried and weighed, examined microscopically, and analyzed for protein; or filtered, washed, and analyzed for protein. Method is applicable to meat scrap, meat and bone scrap, digester tankage, fish meal, whale meal, blood meal, hydrolyzed feather meal, and poultry by-product meal.

22.026 APPARATUS

(a) *Centrifuge.*—Capable of at least 1750 rpm with conical bottom tubes of 150 ml capacity. If necessary, soln may be centrifuged in 50 ml tubes.

(b) *Agitator.*—*See* Fig. 40. Continuous, slow speed (15 rpm), end-over-end type, to operate inside incubator at $45 \pm 2°$ and carry 8 oz screw-cap prescription bottles. Scale diagram or complete agitator (with bottles) available from Moorman Mfg. Co., Quincy, Ill. at cost ($35). Bottles, 8 oz GXR-8 Phoenix prescription; also available from Armstrong Cork Co., Lancaster, Pa. Stirring or reciprocating (shaking) type cannot be used because solid particles collect on sides of container and do not contact pepsin soln.

(c) *Glass fiber filter paper.*—Reeve Angel No. 934-AH, or equiv., 2.4 cm diam. For indigestible residues.

22.027 REAGENTS

(a) *Pepsin soln.*—0.2% pepsin (1:10,000) in 0.075*N* HCl. Prep. dil. HCl by dilg 6.1 ml HCl to 1 L with H_2O. Add pepsin just before use, stirring until completely dissolved.

(b) *Alcohol.*—Denatured is satisfactory.

(c) *Filter aid.*—Diatomaceous earth type such as Hyflo Super-Cel (Johns-Manville, Box 60, New York 16, N. Y.).

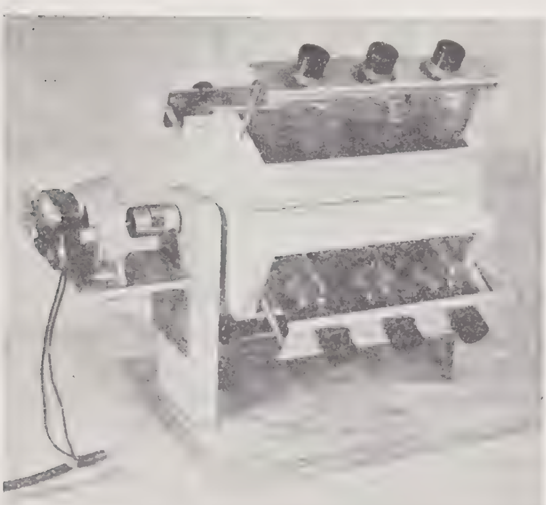

FIG. 40.—AGITATOR

22.028 EXTRACTION

Ext. sample, ground in Wiley mill to pass 2 mm screen, by one of following methods:

(a) *By extraction.*—Prep. extn thimble from 11 cm Whatman No. 2 paper, or equiv., as follows: Fold paper in half; straighten paper and refold at right angles to first fold; turn paper over and repeat process with folds at 45° to original fold; while holding creased paper in one hand, place short test tube (6–8 mm smaller in diam. than extractor sample holder or cup in which thimble is to be used) at its center; fold along natural crease lines to form 4-pointed star around tube; and wrap points in same direction around tube to complete thimble.

Weigh 1.000 g sample into thimble and ext. 1 hr with ether at condensation rate of 3–4 drops /sec. (If Soxhlet is used, top of thimble should extend above siphon tube to avoid loss of solid particles. If paper contg sample is totally submerged in siphon cup, sample must be completely wrapped in paper.) Observe ether ext. to det. that no solid particles were carried into solvent beaker. If approx. fat content is desired, evap. ether, and dry and weigh residue. Remove paper from sample container or cup and let dry at room temp. Unfold and brush defatted sample quantitatively into digestion bottle, avoiding contamination by brush bristles or filter paper fibers. Proceed as in **22.029**.

(b) *By centrifuging.*—Stir 1.000 g sample thoroly with 10 ml ether in 15 ml centrifuge tube and centrifuge 5 min. at 1750 rpm or greater. Decant ether and repeat extn with three 5 ml portions ether. If approx. fat content is desired, combine ether exts, evap., dry, and weigh residue. Proceed as in **22.029**.

22.029 PEPSIN DIGESTION

Transfer defatted sample quantitatively to 8 oz agitator bottle. Add 150 ml freshly prepd pepsin-HCl soln, prewarmed to 42–45°. Stopper bottle, clamp in agitator, and incubate with continuous agitation 16 hr at 45°.

22.030 TREATMENT OF RESIDUE

For indigestible residue, indigestible protein, and microscopic examination of residue.—Transfer contents of agitator bottle to centrifuge tube and centrifuge 5 min. at 1750 rpm or greater. If 150 ml tube is unavailable, centrifuge in 50 ml portions, collecting entire residue in same tube. De-

cant supernatant, rinse agitator bottle twice with 15–20 ml portions warm H_2O, and add rinse H_2O to residue in tube. Stir well, centrifuge, and decant wash H_2O. Wash residue once more with warm H_2O and twice with alcohol. Resuspend residue in 5–10 ml alcohol and filter quantitatively with gentle suction thru weighed No. 4 gooch crucible contg glass fiber filter paper. (Prep. crucible by inserting paper, washing twice with warm H_2O, and twice with alcohol with gentle suction. Dry in oven 30 min. at 100–110°, cool, and weigh.) Wash indigestible residue with alcohol and suck dry. Dry in oven 30 min. at 110°. Cool, weigh, and calc. indigestible residue. Examine microscopically, if desired. Transfer residue and pad quantitatively to Kjeldahl flask and det. crude protein by **2.036.**

For indigestible protein only.—After incubation add ca 1 g filter aid to digestion mixt. in agitator bottle. Filter quantitatively with gentle suction thru Whatman No. 2 paper or thru gooch with glass paper or asbestos pad. Wash 3 times with warm H_2O. Transfer paper contg moist residue to Kjeldahl flask and det. crude protein as in **2.036.**

22.031 CALCULATIONS

Calc. (1) % indigestible residue and (2) % indigestible protein from above on original sample basis; (3) calc. total protein from **2.036.** % crude protein in indigestible residue = (2) × 100/(1); % of crude protein content of sample not digested = (2) × 100/(3); % of crude protein content of sample digested = 100 − % not digested.

Crude Fat or Ether Extract

Direct Method—Official

22.032 REAGENT

Anhydrous ether.—Wash commercial ether with 2 or 3 portions of H_2O, add solid NaOH or KOH, and let stand until most of H_2O is abstracted from the ether. Decant into dry bottle, add small pieces of carefully cleaned metallic Na, and let stand until H evolution ceases. Keep ether, thus dehydrated, over metallic Na in loosely stoppered bottles.

22.033 DETERMINATION

Ext. ca 2 g sample, dried as in **22.003** or **22.007,** with anhyd. ether. Use thimble with porosity permitting rapid passage of the ether. Extn period may vary from 4 hr at condensation rate of 5–6 drops/sec. to 16 hr at 2–3 drops/sec. Dry ext. 30 min. at 100°, cool, and weigh.

22.034 *Indirect Method—Official*

Det. moisture as in **22.003** or **22.007;** then ext. dried substance as in **22.033,** and dry again. Report loss in wt as ether ext.

22.035 *In Baked Dog Food (12)—Official*

Place 2 g ground, well-mixed sample in Mojonnier fat-extn tube, add 2 ml alcohol to prevent lumping on addn of acid, and shake to moisten all particles. Add 10 ml HCl (25+11), mix well, and set tube 30–40 min. in H_2O bath at 70–80°, shaking frequently. Fill to within 1–2 ml of mark with alcohol and cool. (Level of liquid should be in neck of Mojonnier tube just below pouring-off level.)

Add 25 ml ether, stopper with glass, cork, Neoprene, or good quality rubber stopper thoroly cleaned with alcohol, and shake vigorously 1 min. Release pressure carefully so that no solvent is lost. Wash adhering solvent and fat from stopper back into extn tube with few ml redistd petr. ether (b.p. <60°). Add 25 ml redistd petr. ether, stopper, and shake vigorously 1 min. Let stand until upper liquid is practically clear or centrifuge 20 min. at ca 600 rpm. Pour as much of ether-fat soln as possible thru filter consisting of cotton pledget packed just firmly enough in funnel stem to let ether pass freely into 150 ml beaker contg several glass beads. Rinse lip of tube with few ml petr. ether. Re-ext. liquid remaining in tube twice, each time with only 15 ml of each ether, shaking 1 min. after addn of each ether. Draw off clear ether soln thru filter into same beaker as before, and wash tip of tube, stopper, funnel, and end of funnel stem with few ml of mixt. of the 2 ethers (1+1). Evap. slowly on steam bath.

Redissolve dried fat residue in four 10 ml portions Et ether, filtering each portion thru small fat-free paper into 100 ml beaker, contg few glass beads, that has been predried at 100°, cooled in air, and then weighed against counterpoise treated similarly. Use fifth 10 ml portion ether for rinsing paper and funnel. Evap. ether on steam bath, dry in 100° oven 90 min., cool in air, and as soon as room temp. is reached, weigh against counterpoise treated similarly. Correct this wt by blank detn on reagents used.

22.036 *In Dried Milk Products (13)—Official*

Proceed as in **15.108(b)** and **15.109,** using 8.5 ml H_2O and 1.5 ml NH_4OH.

22.037 *In Fish Meal (14)—First Action*

Weigh 4–5 g sample to nearest 0.01 g into Alundum or paper extn thimble, cover with light layer of cotton, and ext. with acetone in continuous extractor 16 hr. Distill off acetone until vol. in flask is 10–15 ml, transfer to 100 ml tared beaker, washing flask free of all oil with fresh acetone, and evap. with current of warm air. (Convenient method is to place flask on warm surface,

e.g., over steam radiator, in front of small elec. fan.) When no H_2O or acetone can be observed, place beaker in vac. oven at 80° and apply 24–25″ vac. 1 hr. Transfer to desiccator, cool, and weigh.

Transfer extd meal residue from thimble to 150 ml beaker. Remove any remaining solvent by heating on warm surface and then add 60 ml 4*N* HCl. Digest 1 hr at or near b.p. on hot plate, stirring occasionally with glass rod and adding H_2O as needed to maintain vol. in beaker. (Complete removal of acetone is necessary before this digestion, otherwise vaporization of solvent will carry meal particles over side of vessel onto hot plate.) Filter thru 12.5 cm fluted paper. Wash residue on filter until acid-free, using Me red on portions of filtrate to follow progress of washing. Place filter and meal in 150 ml beaker and dry 1 hr in air oven at 80–90°. Transfer filter and contents to thimble and ext. 16 hr with acetone. Remove solvent and weigh ext. as above. Sum of wts of exts = total fat.

Crude Fiber (*15*)—First Action

22.038 REAGENTS

(**a**) *Sulfuric acid soln.*—0.255*N*. 1.25 g H_2SO_4/100 ml.

(**b**) *Sodium hydroxide soln.*—0.313*N*. 1.25 g NaOH/100 ml, free, or nearly so, from Na_2CO_3.

Concn of these solns must be accurately checked by titrn.

(**c**) *Asbestos.*—Gooch grade, medium fiber, acid-washed, and ignited is usually satisfactory but should be tested for chemical stability and filtering speed before use. Digest on steam bath or at equiv. temp. at least 8 hr with ca 5% NaOH soln and wash thoroly with hot H_2O; then digest in similar manner 8 hr with HCl (1+3) and again wash thoroly with hot H_2O. Dry, and ignite at bright red heat.

22.039 APPARATUS

(**a**) *Condenser.*—Use condenser that maintains constant vol. of soln thruout digestion.

(**b**) *Digestion flasks.*—Use digestion flasks of such size and shape that soln is not $<1''$ nor $>1.5''$ in depth. 700–750 ml erlenmeyer is recommended.

(**c**) *Filtering cloth.*—Use filtering cloth of such character that no solid matter passes thru when filtering is rapid. (Retention may be tested by passing filtrate thru gooch.) Butcher's linen or dress linen with ca 45 threads/inch or No. 40 filtering cloth made by National Filter Media Corp., New Haven 14, Conn., or its equiv., may be used.

22.040 DETERMINATION

Ext. 2 g dry material with ether, or use residue from ether ext. detn (**22.033** or **22.034**), and transfer residue, together with ca 0.5 g asbestos, to digestion flask. (If residue from the ether ext. is used and proper quantity of asbestos has already been added, further addn is unnecessary.) If material is difficult to wet, add 3 drops dild *Dow-Corning Antifoam A emulsion* (1+3). Add 200 ml of the boiling H_2SO_4 soln, immediately connect digestion flask with condenser, and heat. (Contents of flask must come to boiling within 1 min. and boiling must continue briskly exactly 30 min.) Rotate flask frequently until sample is thoroly wetted. Take care to keep material from remaining on sides of flask out of contact with soln. (Blast of air conducted into flask serves to reduce frothing of liquid.)

After 30 min. remove flask, immediately filter thru linen in fluted funnel, and wash with boiling H_2O until washings are no longer acid. Bring quantity of the NaOH soln to boiling and keep at this temp. under reflux condenser until used. Wash charge and asbestos back into flask with 200 ml of the boiling NaOH soln, using wash bottle marked to deliver 200 ml. (Boiling NaOH soln is conveniently transferred to wash bottle by bent tube thru which liquid is forced by blowing into tube connected with top of reflux condenser attached to NaOH flask.) Connect flask with reflux condenser and boil exactly 30 min., timing boiling with alkali so that contents of different flasks reach b.p. ca 3 min. apart, which permits enough time for filtration.

After 30 min. remove flask and immediately filter thru gooch prepd with asbestos mat, thru Alundum crucible, or thru filtering cloth in fluted funnel. If filtering cloth is used, thoroly wash residue with boiling H_2O and transfer to gooch prepd with thin but close layer of ignited asbestos. For materials difficult to filter, after 30 min. boiling remove flask and immediately filter thru filtering cloth in fluted funnel using vac., and wash with hot 10% K_2SO_4 soln. The K_2SO_4 soln may be added during filtering whenever filtration becomes difficult. Return residue to digestion flask, thoroly washing all residue from cloth with the hot K_2SO_4 soln. Filter into gooch prepd with thin but close layer of ignited asbestos.

After thoro washing with boiling H_2O, wash with ca 15 ml alcohol. Dry crucible and contents at 110° to constant wt. Cool in efficient desiccator and weigh. Ignite contents of crucible in elec. muffle or over Meker burner at dull red heat until carbonaceous matter is consumed (ca 20 min.). Cool in desiccator and weigh. Report loss in wt as crude fiber.

22.041 Reducing Sugars (*16*)—Official

Place 10 g sample in 250 ml vol. flask. If material is acid, neutralize by adding 1–3 g $CaCO_3$. Add 125 ml 50% alcohol by vol., mix thoroly, and boil on steam bath or by partially immersing

flask in H_2O bath at 83–87° 1 hr, using small funnel in neck of flask to condense vapor. Cool and let mixt. stand several hr, preferably overnight. Dil. to vol. with neutral 95% alcohol, mix thoroly, and let settle or centrifuge 15 min. at 1500 rpm and decant supernatant closely. Pipet 200 ml supernatant into beaker and evap. on steam bath to 20–30 ml. Do not evap. to dryness. Little alcohol in residue does no harm.

Transfer to 100 ml vol. flask and rinse beaker thoroly with H_2O, adding rinsings to flask. Add enough *satd neutral $Pb(OAc)_2$ soln* (ca 2 ml) to produce flocculent ppt, shake thoroly, and let stand 15 min. Dil. to mark with H_2O, mix thoroly, and filter thru dry paper. Add enough anhyd. Na_2CO_3 or K oxalate to filtrate to ppt all Pb, again filter thru dry paper, and test filtrate with little anhyd. Na_2CO_3 or K oxalate to make sure that all Pb has been removed.

Proceed as in **29.039**, using 25 ml aliquot (representing 2 g sample). Express results as dextrose or invert sugar.

22.042 **Sucrose** (*16*)—**Official**

Place 50 ml prepd soln, **22.041**, in 100 ml vol. flask, add piece of litmus paper, neutralize with HCl, add 5 ml HCl, and let inversion proceed at room temp. as in **29.026(c)**. When inversion is complete, transfer soln to beaker, neutralize with Na_2CO_3, return soln to 100 ml flask, dil. to mark with H_2O, filter if necessary, and det. reducing sugars in 50 ml of the soln (representing 2 g sample) as in **22.041**. Calc. results as invert sugar. [% total sugar after inversion − % reducing sugars before inversion (both calcd as invert sugar)] ×0.95 = % sucrose.

Because insol. material of grain or cattle food occupies some space in flask as originally made up, correct by multiplying all results by factor 0.97, as results of large number of detns on various materials show av. vol. of 10 g material to be 7.5 ml.

Starch

(Intended only for such materials as raw starch, potatoes, etc., including as starch the pentosans and other carbohydrate bodies that undergo hydrolysis and are converted into reducing sugars on boiling with HCl.)

22.043 *Direct Acid Hydrolysis—Official*

Stir weighed sample, representing 2.5–3 g dry material, 1 hr in beaker with 50 ml cold H_2O. Transfer to filter and wash with 250 ml cold H_2O. Heat insol. residue 2.5 hr with 200 ml H_2O and 20 ml HCl (sp. gr. 1.125) in flask provided with reflux condenser. Cool, and nearly neutralize with NaOH. Transfer to 250 ml vol. flask, dil. to mark, filter, and det. dextrose in aliquot of filtrate as in **29.039**. Wt dextrose obtained ×0.90 = wt starch (*17*).

Diastase Method with Subsequent Acid Hydrolysis—Official

22.044 REAGENT

Malt extract.—Use clean, new barley malt of known efficacy and grind only as needed. Grind well, but not so fine that filtration is greatly retarded. Prep. infusion of freshly ground malt just before use. For every 80 ml malt ext. required, digest 5 g ground malt with 100 ml H_2O, at room temp., 2 hr, or 20 min. if mixt. can be stirred by elec. mixer. Filter to obtain clear ext., refiltering first portions of filtrate if necessary. Mix infusion well.

22.045 DETERMINATION

Ext. quantity of sample (ground to impalpable powder and representing 4–5 g dry material) on close texture filter with five 10 ml portions ether; wash with 150 ml alcohol, 10% by vol., and then with few ml 95% alcohol. Place residue in beaker with 50 ml H_2O, immerse beaker in boiling H_2O, and stir constantly 15 min., or until all starch is gelatinized; cool to 55°, add 20 ml of the malt ext., and hold at this temp. 1 hr. Heat again to boiling few min., cool to 55°, add 20 ml of the malt ext., and hold at this temp. 1 hr, or until residue treated with I soln shows no blue color upon microscopic examination. Cool, dil. to 250 ml, and filter.

Place 200 ml filtrate in flask, add 20 ml HCl (sp. gr. 1.125), connect with reflux condenser, and heat in boiling H_2O bath 2.5 hr. Cool, nearly neutralize with 10% NaOH soln, finish neutralization with Na_2CO_3 soln, and dil. to 500 ml. Mix soln thoroly, pour thru dry filter, and det. dextrose in aliquot as in **29.039**. Conduct blank detn on same vol. of malt ext. as used with sample and correct wt dextrose accordingly. Wt dextrose obtained ×0.90 = wt starch.

22.046 *In Presence of Interfering Polysaccharides* (*18*)—*Official*

Weigh 2–6 g (4 g for linseed meal, or 3 g for dried apple pomace, have been found satisfactory) well-mixed sample, prepd to pass freely thru sieve not <No. 40, using smaller charges with materials contg much gel-forming substance. (Wt starch in charge must not be >1.5 g.) Transfer to dry 12.5–15 cm close-texture, rapid filter paper in glass funnel and ext. with 5 successive portions of ether, taking for each portion more than enough to cover charge and using watch glass to retard evapn.

After completing ether extn, let ether evap. and then ext. charge with 300 ml dil. alcohol. Concn of alcohol may be varied somewhat to suit material under examination. For linseed meal use

35% alcohol (by vol.) and for dried apple pomace use 25% alcohol. Follow this with several filterfuls of 95% alcohol and finish leaching operations with second ether extn. Conduct control detn also, preferably in duplicate, using paper extd with alcohol and same quantity of H_2O and malt ext. as in detn. (It is convenient to let charge stand overnight at this point to let ether and alcohol evap., as alcohol must be absent when malt digestion starts; or dry charge at ca 75° until alcohol-free.)

Transfer as much dry material as possible from filter into glass mortar and pulverize all lumps. Transfer both filter paper and sample to 500 ml vol. flask, add 20–30 ml H_2O, and thoroly wet material by vigorous shaking. If more cold H_2O is needed to make material more fluid, calc. quantity of hot H_2O to be added accordingly, so that total vol., allowing for 40 ml malt soln, will be not >200 ml. Let stand few min., add 100 ml actively boiling H_2O, and thoroly gelatinize in boiling H_2O bath.

Cool to 50° or lower, add 20 ml malt extract, **22.044,** to both controls and samples, and place flasks in temp.-controlled H_2O bath. Keeping mash thoroly mixed, gradually raise temp. to 70° in 20–30 min. Keep 30 min. at 70°, stirring mixts from time to time; then increase temp. to 80°, and keep at that temp. 10 min. Finally heat to b.p. Keep mixts well stirred. Cool contents of flasks and H_2O bath to 55°. Add 20 ml of the malt ext., mix well, and hold 1 hr at 55°, stirring ca once every 10 min. After digestion increase temp. rapidly to >80°.

Measure 316 ml alcohol and add, little at time, to contents of flask, with thoro shaking between addns. Cool to room temp. and adjust vol. with H_2O so that total liquid vol. is 500 ml, allowing for vol. occupied by charge by adding 3 ml H_2O for every 4 g charge present after bringing contents to 500 ml mark. (Detn may be interrupted at this stage for several days, but vol. should be readjusted if evapn occurs.) Mix thoroly, breaking up any ropy coagulum as much as possible by pouring back and forth from one large beaker to another. Filter thru dry paper. Test solid residue for starch, either microscopically or by I color test, after elimination of alcohol and gelatinization with H_2O. (If more than merest trace of starch is found, reject entire detn.) Evap. exactly 200 ml filtrate on steam bath to 15–20 ml, or until practically alcohol-free. Do not evap. to dryness.

Transfer aq. residue of starch conversion products to 200 ml vol. flask with hot H_2O, using policeman to recover any dextrin that may be present. Let cool somewhat, and dil. to 200 ml. Transfer to suitable digestion flask, add 20 ml HCl (sp. gr. 1.125), made by dilg 68 ml HCl (sp. gr. 1.19, or 37% HCl) to 100 ml, and connect flask with reflux condenser. Heat in boiling H_2O bath 2.5 hr. Cool, and for samples of linseed meal or other material yielding solns which at this stage need further purification, add not >1 ml *10% soln of phosphotungstic acid in 1% HCl.* Mix, and let stand at least 15 min.

Transfer to 250 ml vol. flask, dil. to mark, mix well, and filter thru dry paper. Partially neutralize 200 ml filtrate, while stirring, by adding 10 ml NaOH soln (44 g NaOH/100 ml H_2O) and nearly complete neutralization with little powd. anhyd. Na_2CO_3. Transfer to 250 ml vol. flask with H_2O, cool to room temp., dil. to mark, and mix thoroly. Filter, if necessary, and det. dextrose in 50 ml aliquot filtrate gravimetrically as in **29.039.** Correct wt dextrose obtained by subtracting wt dextrose found for same aliquot of the malt control, and multiply corrected wt dextrose by 0.90 to obtain wt starch.

$$\text{Aliquots: Charge} \times \frac{200}{500} \times \frac{200}{250} \times \frac{50}{250},$$

$$\text{or} \quad \text{Charge} \times 0.064.$$

22.047 *In Condensed or Dried Milk Products—Qualitative Test (19)—Official*

Mix ca 2 g sample with 100 ml H_2O and boil mixt. 2 min. Place few ml cooled mixt. on spot plate or in test tube and add drop I-KI test soln (0.05 g I and 0.2 g KI dissolved in 15 ml H_2O). If starch is present, mixt. turns blue.

Pentosans (*20*)—Official

22.048 REAGENTS

(**a**) *Dilute hydrochloric acid.*—12% by wt. To 1 vol. HCl add 2 vols H_2O. Det. % acid by titrn against std alkali and adjust to proper concn by diln or addn of more HCl, as necessary.

(**b**) *Phloroglucin.*—Dissolve small quantity phloroglucin in few drops Ac_2O, heat almost to boiling, and add few drops H_2SO_4. Violet color indicates presence of diresorcin. A phloroglucin that gives more than faint color may be purified as follows: Heat, in beaker, ca 300 ml of the dil. HCl and 11 g commercial phloroglucin, added in small quantities at time and stirred constantly until nearly dissolved. Pour hot soln into enough of the dil., cold HCl to make 1500 ml. Let stand at least overnight, preferably several days, to permit diresorcin to crystallize. Filter immediately before use. Yellow tint does not interfere with its usefulness. In using, add, to distillate, vol. contg required quantity of phloroglucin.

22.049 DETERMINATION

Place in 300 ml distn flask such quantity of sample, 2–5 g, that wt phloroglucide obtained

will be not >0.300 g, together with 100 ml of the dil. HCl and several pieces of recently ignited pumice stone. Place flask on wire gauze, connect with condenser, and heat, rather gently at first, and then regulate so as to distill 30 ml in ca 10 min. Filter distillate thru small paper. To replace the 30 ml distd add like quantity of the dil. acid from separator so as to wash down particles adhering to sides of flask, and continue process until distillate totals 360 ml. To total distillate gradually add quantity of phloroglucin dissolved in the dil. HCl and thoroly stir resulting mixt. (Quantity of phloroglucin used should be about double that of furfural expected. Soln turns yellow, then green, and soon there appears amorphous greenish ppt that grows darker rapidly, till it becomes almost black.) Dil. to 400 ml with the dil. HCl and let stand overnight.

Collect amorphous black ppt in weighed gooch with asbestos mat, wash carefully with 150 ml H_2O so that the H_2O is not entirely removed from crucible until very last, and dry 4 hr at temp. of boiling H_2O. Cool and weigh in weighing bottle. Increase in wt is considered to be furfural phloroglucide. To calc. furfural, pentoses, or pentosans from phloroglucide, use following formulas given by Kröber:

(1) For wt phloroglucide, designated by a in following formulas, <0.03 g:

Furfural $= (a + 0.0052) \times 0.5170.$

Pentoses $= (a + 0.0052) \times 1.0170.$

Pentosans $= (a + 0.0052) \times 0.8949.$

In preceding and also in following formulas, factor 0.0052 represents wt phloroglucide that remains dissolved in the 400 ml acid soln.

(2) For wt phloroglucide, a, *between* 0.03 and 0.300 g, use Kröber's table, **43.020**, or following formulas (*21*):

Furfural $= (a + 0.0052) \times 0.5185.$

Pentoses $= (a + 0.0052) \times 1.0075.$

Pentosans $= (a + 0.0052) \times 0.8866.$

(3) For wt phloroglucide, a, >0.300 g, use following formulas:

Furfural $= (a + 0.0052) \times 0.5180.$

Pentoses $= (a + 0.0052) \times 1.0026.$

Pentosans $= (a + 0.0052) \times 0.8824.$

22.050 Galactan—First Action

Ext. sample, representing 2.5–3 g dry material, on hardened filter with five 10 ml portions ether; place extd residue in beaker, ca 5.5 cm diam. and 7 cm deep; add 60 ml HNO_3 (sp. gr. 1.15); and evap. on steam bath to 20 ml. Let stand 24 hr, add 10 ml H_2O, and let stand another 24 hr.

Filter, wash impure mucic acid crystals with 30 ml H_2O to remove as much HNO_3 as possible, and return filter and contents to original beaker. Add 30 ml *$(NH_4)_2CO_3$ soln* (1 part $(NH_4)_2CO_3$, 19 parts H_2O, and 1 part NH_4OH) and heat mixt. in H_2O bath, at 80°, 15 min., with constant stirring. (The $(NH_4)_2CO_3$ combines with mucic acid, forming sol. NH_4 mucate.) Wash paper and contents several times with hot H_2O by decantation, passing washings thru filter paper; finally transfer residue to paper, and wash thoroly. Evap. filtrate to dryness on H_2O bath, avoiding unnecessary heating, which causes decomposition; add 5 ml of the HNO_3, stir mixt. thoroly, and let stand 30 min.

Collect pptd mucic acid on weighed gooch or other filter; wash with 10–15 ml H_2O, then with 60 ml alcohol, and then a number of times with ether; dry at temp. of boiling H_2O 3 hr; and weigh. Multiply wt mucic acid by 1.33 to convert to galactose, and by 1.20 to convert to galactan.

22.051 Water-Soluble Acidity (*22*)—Official

Weigh 10 g sample into shaking bottle, add 200 ml H_2O, and shake 15 min. Filter ext. thru folded paper and take 20 ml aliquot (1 g sample). Dil. with 50 ml H_2O and titr. with 0.1N NaOH, using phthln. Report results in terms of ml 0.1N NaOH required to neutralize ext. from 1 g material.

22.052 Salt (*23*) (Qualitative)—Official

Transfer 2 ml 5% $AgNO_3$ soln to small test tube 1 cm i.d. Carefully add to this liquid equal vol. of sample, ground to pass 1 mm sieve, so that most of sample floats or remains above liquid. Gradually incline tube so that liquid is absorbed. White patches of AgCl appear wherever minutest crystal of NaCl comes in contact with soln. These patches may easily be observed with lens or even with naked eye.

22.053 Rice Hulls in Rice Bran (*24*)—Official

Grind small portion of well-mixed sample until it passes thru No. 60 sieve. Weigh 4 mg on slide ruled with parallel lines 1/20″ apart or transfer to ruled slide after weighing. Add just enough chloral hydrate soln (1+1) to fill in under cover-glass, which, preferably, should be square (ca 22 mm). After placing cover-glass, warm gently, but do not boil, to eliminate starch masses and clear tissues. Count particles of hull tissue, using microscope at ca 90×. High refraction and yellowish green color of hull particles help distinguish small pieces not easily recognized by their structure. (To avoid duplicate counting, disregard particles that extend over upper line of strip.) Compare results with those obtained on stds contg known quantities of hulls.

22.054 Oat Hulls in Oats and Oat Feeds (*25*)—Official

(Results are only approximate)

Place in 1 L beaker 800 ml H_2O and 2 g sample, previously ground to pass thru sieve having circular openings 1 mm diam. Stir vigorously to obtain centrifugal effect, let stand 5 min., and decant supernatant carefully, retaining so far as possible all hull particles. Repeat procedure several times until supernatant becomes clear, or nearly so, and then transfer residue with aid of 150 ml H_2O to 300 ml beaker. Add 5 drops HCl and boil 2 min., stirring constantly. Transfer to original beaker with aid of 500 ml H_2O, stir, and let stand until supernatant is clear. Siphon off liquid with 3–4 mm bore rubber tubing, using pinch clamp to control flow so that practically all liquid is removed. (Tilting beaker also helps to obtain this result.) If deposit forms on standing, siphon again. Transfer hulls with aid of H_2O to paper filter, wash several times with alcohol, and dry to constant wt at room temp. When dry, carefully remove hulls from paper, using small stiff brush if necessary, and weigh. (Weighed gooch may be used instead of paper filter.) Wt hulls $\times 50 = \%$ hulls in sample.

22.055 Grit in Poultry and Similar Feeds (*26*)—Official

Place 2 g prepd sample, **22.002,** thoroly mixed, in ca 30 ml evapg dish. Add ca 5 ml $CHCl_3$ and mix gently with glass rod until liquid contacts entire sample. Brush particles on rod into dish, and after pushing all particles down into the $CHCl_3$ with 25 mm circular or square cover-glass, use glass to skim off or pull floating material over top of dish, taking care not to dip cover-glass deep enough to disturb grit at bottom of dish. After skimming until surface of $CHCl_3$ is nearly clear, slowly pour supernatant into second evapg dish. Wash sides of dish with few ml more $CHCl_3$ and repeat skimming and decanting operation until no floating particles remain (10–15 ml $CHCl_3$). When only grit remains, let last traces of $CHCl_3$ evap. spontaneously, and weigh. Wt residue $\times 50 = \%$ grit. After weighing, examine residue for impurities. Also pour out $CHCl_3$ washings collected in second dish and observe whether any grit has been transferred to it during process.

If sample contains NaCl, remove from grit by washing with H_2O. Identify bone in grit by charring. If pelleted feeds or feeds contg molasses are being examined, disintegrate in cold H_2O and dry with alcohol or ether.

22.056 Bone in Meat Scrap or Tankage (*27*)—Official

Sep. bone as in **22.055.** In some instances it may be necessary, after first washing with $CHCl_3$ to rub remaining bone residue with glass rod or small pestle in order to bring some of adhering particles to surface of $CHCl_3$.

Calcium

22.057 *Method I. (28)—Official*

(Applicable to mineral feeds only)

Weigh 2 g finely ground sample into SiO_2 or porcelain dish and ignite in muffle to C-free ash, but avoid fusing. Boil residue in 40 ml HCl (1+3) and few drops HNO_3. Transfer to 250 ml vol. flask, cool, dil. to mark, and mix thoroly. Pipet 25 ml clear liquid into beaker, dil. to ca 100 ml, and add 2 drops Me red, **2.034(i)**. Add NH_4OH (1+1) dropwise to pH 5.6, as shown by intermediate brownish-orange color. If overstepped, add with dropper HCl (1+3) to orange point. Add 2 drops HCl (1+3). Color should now be pink (pH 2.5–3.0), not orange. Dil. to ca 150 ml, bring to boil, and add slowly with constant stirring 10 ml hot satd (4.2%) soln $(NH_4)_2C_2O_4$. If red color changes to orange or yellow, add HCl (1+3) dropwise until color again changes to pink. Let stand overnight for ppt to settle. Filter supernatant thru quant. paper, gooch, or fritted glass filter (fine Pyrex is preferable), and wash ppt thoroly with NH_4OH (1+50). Place paper or crucible with ppt in original beaker, and add mixt. of 125 ml H_2O and 5 ml H_2SO_4. Heat to 70° or above and titr. with $0.1N$ $KMnO_4$ soln to first slight pink color. Presence of paper may cause color to fade in few sec. Correct for blank and calc. % Ca.

Method II. (29)—Official

22.058 Preparation of Solution

(**a**) Weigh 2.5 g sample into 500 or 800 ml Kjeldahl flask. Add 20–30 ml HNO_3 and boil gently 30–45 min. to oxidize all easily oxidizable matter. Cool soln somewhat and add 10 ml 70–72% $HClO_4$. (Observe precautions, p. xviii.) Boil very gently, adjusting flame as necessary, until soln is colorless or nearly so and dense white fumes appear. Use particular care not to boil to dryness (Danger!) at any time. Cool slightly, add 50 ml H_2O, and boil to drive out any remaining NO_2 fumes. Cool, dil., filter into 250 ml vol. flask, dil. to vol., and mix thoroly.

(**b**) Weigh 2.5 g finely ground sample into SiO_2 or porcelain dish and ignite as in **22.010**. Add 40 ml HCl (1+3) and few drops HNO_3 to residue, boil, transfer to 250 ml vol. flask, cool, dil. to mark, and mix thoroly.

22.059 Determination

Pipet suitable aliquot of clear soln, **22.058(a)** or (**b**), into beaker, dil. to 100 ml, and add 2 drops Me red, **2.034(i)**. Continue as in **22.057**, beginning

"Add NH_4OH (1+1) dropwise . . ." except to use $0.05N$ $KMnO_4$.

(100 ml is suitable aliquot of sample soln for grain feeds; for mineral feeds 25 ml aliquot may be taken and titrd with $0.1N$ $KMnO_4$. For suitable precision, size of sample, aliquot, and concn of $KMnO_4$ must be so adjusted that at least 20 ml std $KMnO_4$ soln is consumed.)

22.060 Fluorine—Official

See **24.025–24.031,** especially **24.029.**

22.061 Phosphorus in Feeds *(29)*—**Official**

Using aliquot of soln, **22.058(a)**, proceed as in **2.022(a)**. Calc. as % P.

Cyanogenetic Glucosides in Feeds and Similar Materials *(30)*

22.062 *Qualitative Test—Official*

Prep. Na picrate paper by dipping strips of filter paper into 1% picric acid soln and drying; then dipping into 10% Na_2CO_3 soln and drying. Store these papers in stoppered bottle.

Chop finely small quantity of plant material and place in test tube. Insert piece of the moist Na picrate paper in tube, taking care that it does not come in contact with material. Add few drops $CHCl_3$ and stopper tube tightly. The Na picrate paper gradually turns orange, then brick red, if plant tissue contains cyanogenetic glucosides. (Test is delicate, and rapidity of change in color depends upon quantity of free HCN present. This test works well with fresh plant materials, but relatively dry substances, particularly seeds of various plants, should be ground and moistened with H_2O and allowed to hydrolyze in stoppered test tube contg Na picrate paper. If necessary, small quantity of emulsin may be added.)

Hydrocyanic Acid Formed by Hydrolysis of Glucosides in Beans *(31)*—Official

22.063 *Acid Titration Method*

Place 10–20 g sample, ground to pass No. 20 sieve, in 800 ml Kjeldahl flask, add 100 ml H_2O, and macerate at room temp. 2 hr. Add 100 ml H_2O and steam distill, collecting distillate in 20 ml $0.02N$ $AgNO_3$ acidified with 1 ml HNO_3. Before distg, adjust app. so that tip of condenser dips below surface of liquid in receiver. When 150 ml has passed over, filter distillate thru gooch; wash receiver and gooch with little H_2O; and titr. excess $AgNO_3$ in combined filtrate and washings with $0.02N$ KCNS, using Fe alum indicator. 1 ml $0.02N$ $AgNO_3$ = 0.54 mg HCN.

22.064 *Alkaline Titration Method*

Place 10–20 g sample, ground to pass No. 20 sieve, in 800 ml Kjeldahl flask, add ca 200 ml H_2O, and let stand 2–4 hr. (Autolysis should be conducted with app. completely connected for distn.) Steam distill, collect 150–160 ml distillate in NaOH soln (0.5 g in 20 ml H_2O), and dil. to definite vol.

To 100 ml distillate (it is preferable to dil. to 250 ml and titr. 100 ml aliquot) add 8 ml $6N$ NH_4OH and 2 ml 5% KI soln and titr. with $0.02N$ $AgNO_3$, using microburet. End point is faint but permanent turbidity and may be easily recognized, especially against black background. 1 ml $0.02N$ $AgNO_3$ = 1.08 mg HCN.

22.065 Ferrous Sulfate *(32)*—**Official**

Moisten entire surface of sheet of white glazed paper with 10% $K_3Fe(CN)_6$ soln. Sift portion of sample thru fine sieve (No. 40) so that feed is distributed thinly over entire area. After few moments wash off feed under slow stream of H_2O. Blue speck or spot denotes particle of ferrous salt.

22.066 Copper Sulfate *(32)*—**Official**

Proceed as in **22.065,** except use 10% $K_4Fe(CN)_6$ soln. Brown speck or spot denotes particle of Cu salt.

22.067 Potassium Iodide *(32)*—**Official**

Moisten entire surface of sheet of white glazed paper with mixt. of starch indicator and $Br\text{-}H_2O$ (3 parts former to 1 of latter). Sift portion of sample so that feed is distributed thinly over entire area. Blue color denotes particle of iodide. If extremely small quantity of KI is to be detected, modify procedure by carefully charring 10 g or more of feed, washing residue with small quantity of H_2O, and evapg filtered soln in white evapg dish so that solids are concd on one small spot. When moistened with the starch indicator and $Br\text{-}H_2O$, blue color denotes presence of iodide.

Iodine in Mineral Mixed Feeds—Official

Knapheide-Lamb Method (33)

22.068 REAGENTS

(a) *Reduced phosphoric acid.*—20%. Reduce impurities in H_3PO_4 according to Kendall's method *(34)* by dilg 85% acid with 4 vols H_2O and boiling some time with Al strips.

(b) *Sodium thiosulfate soln.*—$0.005N$. Preferably stdze as follows: Pipet, into beaker, 25 ml soln contg 0.1308 g KI/L and add 200 ml H_2O, 5 ml 20% $NaHSO_3$ soln, and 2 or 3 g NaOH. Neutralize mixt. with sirupy H_3PO_4, add 1 ml excess, and proceed as in regular detn. To calc. mg I to which 1 ml of the $Na_2S_2O_3$ soln is equiv., use following formula: 2.5/ml $Na_2S_2O_3$ soln. (Stdze the $Na_2S_2O_3$ soln same day detn is made.)

22.069 APPARATUS (*35*)

Furnace.—Use sheet Fe cylinder 4″ diam. and 12″ high, with center opening in top to take 100 ml Ni crucible. Suspend 2¾″ circular plate in center of cylinder 3″ below top, for spreading flame, thereby preventing free flame from coming in contact with crucible, and providing uniform heat. Make slot at bottom of cylinder 1″ wide by 3″ high for admitting air and burner tubing, and near top rim make eight ½″ holes to allow for escape of exhaust gases.

22.070 DETERMINATION

Fuse together in 100 ml Ni crucible 20 g NaOH and 10 g KNO_3, and cool. Place evenly on top of fused alkali 1–10 g sample (depending upon its composition and trouble experienced from frothing during fusion) and completely moisten with 5 ml NaOH soln (1+1) and 10 ml 80% alcohol. Place crucible on cold 3-heat hot plate and evap. alcohol at low heat. After 30 min. cautiously increase heat until crucible has been subjected to highest temp. of hot plate for 1.5–2 hr. (Thoro heating at this stage prevents most effervescence of material during fusion.) Then place crucible in above or similar furnace.

To prevent loss, give close attention during fusion to mineral mixts contg charcoal or org. matter because of violent reaction between the C and the KNO_3. If reaction becomes too violent, lift crucible from furnace for moment, and if necessary cool bottom of crucible in H_2O. When mixt. is in quiet state of fusion tip crucible on all sides in open flame to wash down fusion mixt. Add few small KNO_3 crystals until no more gas is liberated by further addns, and again wash down sides of crucible in flame.

Pour melt out onto clean crucible cover to cool, or turn crucible while cooling so that material solidifies on sides. Place cooled melt and crucible in 600 ml beaker, cover with H_2O, and heat below b.p. short time. Let mixt. stand overnight at room temp., rinse off crucible and cover, and remove. In order to neutralize part of alkali and facilitate filtering, add 10 ml 85% H_3PO_4 and place beaker on steam bath 3–4 hr, stirring occasionally to break up mass and insure complete soln of the I. Cool beaker, filter off insol. residue in 10 cm funnel, and wash with cold H_2O into 800 ml beaker, adjusting vol. to 550–600 ml. (Soln should be clear and colorless.)

In order to destroy nitrites, which interfere with titrn with Me orange, add 10 ml 20% $NaHSO_3$ soln, bring soln just to b.p., and cool. Add ca 30 ml 85% H_3PO_4 from buret and few drops Me orange soln; continue addn of H_3PO_4 to neutral color of Me orange, and finally add 1.5 ml H_3PO_4 in excess. (Total quantity H_3PO_4 required is generally not >35 ml, except when presence of considerable C in sample requires use of more KNO_3, which is mainly reduced to carbonate.) Use care not to run appreciably over end point, as excess acid gives low results. However, addn of acid must be fairly rapid, as color of Me orange tends to fade, due to incomplete destruction of nitrites.

After neutralization, add small lump anthracite coal (0.5 cm in diam.) and boil soln at least 20 min., reducing vol. to 400–500 ml. (Boiling is essential to remove all traces of SO_2.) Again cool soln and add Br-H_2O until distinct and permanent yellow color is produced. Boil soln until colorless by reflected light and then exactly 5 min. longer. Add few crystals *salicylic acid* to assure removal of last traces of Br, cool soln, and add 5 ml of the 20% reduced H_3PO_4 and 0.5–1.0 g KI. Titr. soln in usual manner with 0.005*N* $Na_2S_2O_3$, adding starch soln when brown color of liberated I is nearly gone. (Vol. of soln at final titrn should be 400–500 ml.)

22.071 *Elmslie-Caldwell Method* (*36*)

Place sample contg 3–4 mg I in 200–300 ml Ni dish. Add ca 5 g Na_2CO_3, 5 ml NaOH soln (1+1), and 10 ml alcohol, taking care that entire sample is moist. Dry at ca 100° to prevent spattering upon subsequent heating (30 min. is usually enough).

Place dish and contents in furnace heated to 500° and keep at that temp. 15 min. (Ignition of sample at 500° appears to be necessary only to carbonize any sol. org. matter that would be oxidized by Br-H_2O if not so treated. Temp. >500° may be used if necessary.) Cool, add 25 ml H_2O, cover dish with watch glass, and boil gently 10 min. Filter thru 18 cm filter paper and wash with boiling H_2O, catching filtrate and washings in 600 ml beaker (soln should total ca 300 ml). Neutralize to Me orange with 85% H_3PO_4 and add 1 ml excess.

Add excess Br-H_2O and boil soln gently until colorless, and then 5 min. longer. Add few crystals *salicylic acid* and cool soln to ca 20°. Add 1 ml 85% H_3PO_4 and ca 0.5 g KI, and titr. I with 0.005*N* $Na_2S_2O_3$ as in **22.070**.

Acid-Soluble Manganese (*37*)—Official

22.072 REAGENT

Potassium permanganate std soln.—Dissolve 1.4383 g $KMnO_4$ by boiling with H_2O. Dil. to 1 L, let stand several days, and filter thru asbestos pad on gooch. Stdze with Na oxalate (soln should contain 500 ppm Mn). Transfer aliquot contg 20 mg Mn to beaker. Add 100 ml H_2O, 15 ml H_3PO_4, and 0.3 g KIO_4, and heat to b.p. Cool, and dil. to 1 L. Protect from light. Dil. this soln contg 20 ppm Mn with H_2O (previously boiled with 0.3 g

KIO_4/L) to make convenient working stds in range of concns to be compared.

22.073 DETERMINATION

Ash weighed sample, 5–15 g, at dull red heat in porcelain dish. Cool, and add 5 ml H_2SO_4 and 5 ml HNO_3 to ash in dish or to ash transferred to beaker with 20–30 ml H_2O. Evap. to white fumes. If C is not completely destroyed, add further portions of HNO_3, boiling after each addn. Cool slightly, transfer to 50 or 100 ml vol. flask, and add vol. dil. H_3PO_4 soln (8+92) equal to ½ vol. of flask (25 or 50 ml). Cool, dil. to vol., mix, and filter or let stand until clear.

If 50 ml flask was used, pipet 25 ml clear soln into beaker or 50 or 100 ml vol. flask and add 15 ml H_2O. If 100 ml flask was used, pipet 50 ml into beaker or 100 ml flask and add 30 ml H_2O. Heat nearly to b.p., and with stirring or swirling add 0.3 g KIO_4 for each 15 mg Mn present. Keep 30–60 min. at 90–100°, or until color development is complete. Cool, dil. to measured vol. of 50 or 100 ml and mix. Compare with the std $KMnO_4$ soln in colorimeter or in spectrophotometer at 530 mμ. Calc. ppm Mn.

Cobalt (*38*)—Official

22.074 REAGENTS

(a) *Cobalt sulfate soln.*—Do not dry; use as received. Dissolve 0.2385 g $CoSO_4.7H_2O$ in H_2O and dil. to 1 L. 1 ml = 0.05 mg Co. This soln may be dild to suitable concn to prep. std curve.

(b) *Nitroso-R salt soln.*—($C_{10}H_4OH.NO(SO_3Na)_2$). Dissolve 1 g in H_2O and dil. to 500 ml.

(c) *Spekker acid.*—Mix 150 ml 85% H_3PO_4 and 150 ml H_2SO_4, and dil. to 1 L with H_2O.

(d) *Sodium acetate soln.*—Dissolve 500 g $NaOAc.3H_2O$ in H_2O and dil. to 1 L with H_2O.

22.075 PREPARATION OF STANDARD CURVE

To 1, 2, etc., up to 11 ml portions of the std $CoSO_4$ soln in 100 ml vol. flasks add 2 ml Spekker acid, 10 ml nitroso-R salt soln, and 10 ml NaOAc soln. Prep. blank by using 2 ml Spekker acid and 10 ml NaOAc soln, but omitting the nitroso-R salt soln. Bring blank and std solns to boil on hot plate. Add 5 ml HNO_3 and boil solns at least 1, but not >2 min. Cool, and dil. solns to 100 ml.

22.076 DETERMINATION

Ash 2 g sample 2 hr at 600°, transfer to 200 ml vol. flask with 20 ml HCl and 50 ml H_2O, boil 5 min., cool, and dil. to vol. Let soln settle. Pipet suitable aliquot into small flask. For samples contg 0.01–0.2% Co use equiv. of 0.25 g sample. Use more or less according to Co concn expected. Max. quantity Co in sample should be 0.5 mg, since soln no longer appears to follow Beer's law above this concn.

Pass brisk current of *H_2S* thru soln 10 min. Filter directly into 100 ml vol. flask thru Whatman No. 40 paper. Wash with ca 50 ml *1% H_2SO_4 satd with H_2S*. Add 2 small glass beads and boil off H_2S. (Flasks must be given individual attention, as violent bumping may occur.) Shake flasks often. Add 5 ml HNO_3 and boil until nitrous fumes no longer appear. (Take care, as vol. of soln will be low and bumping and spattering may occur. At first indication of this, remove immediately from hot plate.) Small amount HNO_3 remaining will not affect result. Cool, add 2 drops phthln, and take to first faint pink with ca 30% NaOH soln. Immediately add 2 ml Spekker acid followed by 10 ml nitroso-R salt soln and 10 ml NaOAc soln. Bring to vigorous boil, carefully add 5 ml HNO_3, and boil at least 1, but not >2 min. Cool and dil. to vol.

Compare color with std Co solns in colorimeter, using green or No. 54 filter, or in spectrophotometer at 540 mμ. Read color within 2 hr. Report % Co to third decimal place.

Copper (*39*)—Official

22.077 PREPARATION OF STANDARD CURVE

Dissolve 1.9645 g $CuSO_4.5H_2O$ in H_2O and dil. to 500 ml. (1 ml = 1 mg Cu.) Use from 1 to 10 ml of this soln to prep. set of stds in 100 ml Pyrex g-s. vol. flasks. Add 4 ml HCl, dil. to 50 ml, add 5 ml *tetraethylenepentamine*, dil. to mark with H_2O, stopper, and mix thoroly. Prep. blank, using all reagents except Cu. Filter blank and stds before reading color as in **22.078.**

22.078 DETERMINATION

Prep. sample soln as in **22.076,** using 8 g sample. Pipet 50 ml aliquot into 100 ml Pyrex g-s. vol. flask, add 5 ml tetraethylenepentamine, dil. to vol. with H_2O, and mix thoroly. Filter and compare colors within 30 min. in colorimeter (red or No. 66 filter) or read in spectrophotometer at 620 mμ. Report % Cu to third decimal place.

Soluble Chlorine (*40*)—Official

22.079 REAGENTS

(a) *Potassium chloride soln.*—Recrystallize reagent KCl 3 times from H_2O, dry at 110°, and then heat at ca 500° to constant wt. Dissolve 2.1027 g in H_2O and dil. to 1 L. Soln contains 0.001 g Cl/ml.

(b) *Silver nitrate soln.*—Dissolve 5 g $AgNO_3$ in 1 L H_2O and adjust soln so that 1 ml = 1 ml std KCl soln.

(c) *Potassium thiocyanate soln.*—Dissolve 2.5 g KSCN in 1 L H_2O and adjust so that 1 ml = 1 ml std $AgNO_3$ soln. Stdze as in **42.004.**

(d) *Ferric sulfate soln.*—Dissolve 60 g $Fe_2(SO_4)_3$ + Aq in H_2O and dil. to 1 L.

(e) *Ferric sulfate indicator*.—To filtered 25% soln of $Fe_2(SO_4)_3$+Aq add equal vol. HNO_3.

22.080 DETERMINATION

Transfer 3 g sample to 300 ml erlenmeyer. Add 50 ml of the $Fe_2(SO_4)_3$ soln (accurately measured with pipet or other calibrated app.), swirling flask to prevent caking of sample and to facilitate soln of Cl. Add 100 ml (also accurately measured) NH_4OH (1+19). Swirl flask enough to insure soln of Cl and thoro mixing of soln. (Very little swirling is necessary. If soln is agitated by vigorous vertical shaking, filtration will be difficult.) Let mixt. settle 10 min. Filter thru dry 11 cm Whatman No. 41 paper or equiv. Use 50 ml aliquots ($\frac{1}{3}$ of total) on samples low in Cl (0–2% Cl) and 25 ml aliquots ($\frac{1}{6}$ of total) on samples high in Cl (>2%). For mineral and other feeds contg >10% Cl, weigh 1 g and use 15 ml ($\frac{1}{10}$ of total).

If approx. % Cl in sample is not known, take 10 ml aliquot for trial titrn. To this add 10 ml HNO_3 and 10 ml of the $Fe_2(SO_4)_3$ indicator. Dil. to ca 50 ml. Add 0.5 ml of the KSCN soln and immediately add, with stirring, enough $AgNO_3$ soln to entirely eliminate any reddish color. From this titrn calc. vol. $AgNO_3$ soln necessary to ppt all Cl in aliquot to be used, adding excess equal to ca 10% total vol. necessary, altho somewhat greater excess will not affect results. Min. total of 10 ml should be used.

To sample aliquot in 250 ml beaker add 10 ml HNO_3 and 10 ml of the $Fe_2(SO_4)_3$ indicator (or 20 ml soln contg equal vols of these solns). Then add, with stirring, calcd vol. $AgNO_3$ soln. Heat to boiling and cool to room temp., stirring enough to coagulate ppt. (Cooling may be hastened by immersion of beakers in cold H_2O.) Titr. excess $AgNO_3$ with KSCN. End point is indicated by first appearance of reddish tint that persists 15 sec. For accurate work use reference soln contg all ingredients except KSCN. End point is first change in color.

22.081 Drugs in Feeds—*See* **Chapter 33**

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23. Meat and Meat Products

MEAT

23.001 Preparation of Sample—Procedure

To prevent H_2O loss during prepn and subsequent handling do not use small samples. Keep ground material in glass or similar containers with air- and H_2O-tight covers. Prep. samples for analysis in following manner:

(**a**) *Fresh meats, dried meats, cured meats, smoked meats, etc.*—Sep. as completely as possible from any bone; pass rapidly thru food chopper 3 times, mixing thoroly after each grinding; and begin all detns promptly. If any delay occurs, chill sample to inhibit decomposition.

(**b**) *Canned meats.*—Pass entire contents of can thru food chopper, as in (**a**).

(**c**) *Sausages.*—Remove from casings and pass thru food chopper, as in (**a**).

Dry portions of samples of (**a**), (**b**), and (**c**) not needed for immediate analysis, either *in vacuo* <60° or by evapg on steam bath 2 or 3 times with alcohol. Ext. fat from dried product with petr. ether (b.p. <60°) and let petr. ether evap. spontaneously, finally expelling last traces by heating short time on steam bath. Do not heat sample or sepd fat longer than necessary because of tendency to decompose. Reserve fat in cool place for examination as in Chap. **26,** and complete examination before it becomes rancid.

Moisture

23.002 *Drying in Vacuo at 95–100°—Official*

Proceed as in **22.003.** (Not suitable for high fat products such as pork sausage.)

23.003 *Air Drying (1)—First Action*

(**a**) Dry, with lids removed, sample representing ca 2 g dry material 16–18 hr at 100–102° in air oven (mechanical convection preferred). Use covered Al dish at least 50 mm diam. and not >40 mm deep. Cool in desiccator and weigh. Report loss in wt as moisture.

(**b**) Dry, with lids removed, sample representing ca 2 g dry material to constant wt (2–4 hr depending on product) in mechanical convection oven at ca 125°. Use covered Al dish at least 50 mm diam. and not >40 mm deep. Avoid excessive drying. Cover, cool in desiccator, and weigh. Report loss in wt as moisture.

(NOTE: Dried sample is not satisfactory for subsequent fat detn.)

23.004 Added Water in Sausage (*2*)—Procedure

Per cent H_2O added $=(W-4P)/(1-0.01W+0.04P)$; where $W=\%\ H_2O$, and P (% protein) $=6.25\times\%N$, **23.009** (corrected if necessary for protein in added substances such as nonfat dry milk, cereal, soybean flour).

23.005 Crude Fat or Ether Extract—Official

(**a**) Weigh 3–4 g sample by difference into thimble contg small amount of sand or asbestos. Mix with glass rod and place thimble and rod in 50 ml beaker and dry in oven 6 hr at 100–102° or 1.5 hr at 125°. Proceed as in **22.033,** using petr. ether, **10.090,** if desired.

(**b**) Weigh 3–4 g sample by difference into small disposable Al dish, add sand or asbestos, and mix, spreading mixt. on bottom of dish with glass or Al paddle. Dry with paddle as in (**a**). Roll edges of dish and insert with paddle into thimble. Proceed as in **22.033,** using petr. ether, **10.090,** if desired.

23.006 Ash—Official—*See* 29.012 or 29.013

23.007 Salt—First Action

Moisten 2.5–3 g sample in 300 ml flask with excess 0.5*N* $AgNO_3$ soln, **42.025** (5 ml or more, depending on NaCl content of sample). Add 15 ml HNO_3 and boil until meat dissolves (10 min. usually enough). Add concd aq. $KMnO_4$ soln in small portions, boiling after each addn until $KMnO_4$ color disappears and soln becomes colorless or nearly so. Add 25 ml H_2O and boil 5 min. Cool, dil. to ca 150 ml, add 25 ml ether, and shake. Det. Cl as in **18.009.**

23.008 Total Phosphorus—Official

Destroy org. matter as in **2.018**(**c**) or (**d**), and proceed as in **2.019** or **2.022.**

23.009 Nitrogen (*3*)—Official

Proceed as in **2.036,** using ca 2 g fresh sample

Nitrates and Nitrites

Ferrous Chloride Method (4)—First Action

23.010 REAGENTS

(a) *Ferrous chloride soln.*—Dissolve 400 g nails, tacks, or other small pieces Fe in 2 L Florence flask with 1 L HCl, excluding air from flask with stopper equipped with Bunsen valve. When evolution of gas ceases, transfer, and keep soln in completely filled 50 ml g-s. bottles. Use reagent from freshly opened bottles only.

(b) *Sodium nitrate std soln.*—Dissolve 2 g $NaNO_3$ in 1 L recently boiled H_2O. Det. NO in 50 ml soln (equiv. to 0.1 g $NaNO_3$) as in **23.012.**

23.011 APPARATUS

Clamp to stand 500 ml Kjeldahl flask fitted with 2 hole stopper. Thru 1 hole pass stem of 100–125 ml cylindrical separator having glass stopcock, and into other fit delivery tube leading downward at angle from flask into trough contg soln of commercial NaOH (1+1). Terminate upper end of delivery tube just below stopper in flask and place lower end, slightly constricted, bent upward, and covered with rubber tubing to prevent fracture, under surface of the NaOH soln in trough, exit being just below mouth of inverted measuring tube (50 ml plain eudiometer tube) filled with the NaOH soln. (Single coil of Sn tubing fitted into trough and carrying current of cold H_2O greatly facilitates detn.)

23.012 DETERMINATION

Ext. 100 g sample by boiling 6–7 times with successive 35–50 ml portions H_2O, decant exts thru muslin or paper filter into casserole, and evap. combined exts to ca 50 ml. Add 50 ml of the $FeCl_2$ soln and 50 ml HCl (1+2.5) to Kjeldahl flask, close separator stopcock, move end of delivery tube so that escaping air will not pass into measuring tube, and boil contents of flask until air is completely expelled. Place exit end of delivery tube beneath measuring tube and boil contents of flask 1 min. longer to assure absence of air. Add 50 ml std $NaNO_3$ soln to flask, little at time thru separator, continuously boiling contents of flask to force NO gas into measuring tube. Finally rinse separator 3 or 4 times with 5–10 ml recently boiled H_2O, adding rinsings to contents of evolution flask as described above.

When gas evolution ceases, cover opening of measuring tube with porcelain crucible, using tongs, and carefully transfer tube to tall glass jar contg NaOH soln (1+1), kept at room temp. When contents of tube reach temp. of surrounding caustic soln (10–15 min.), read vol. NO with tube in such position that soln level within tube coincides with level outside. Calc. % nitrates and nitrites as $NaNO_3$ from vol. NO obtained from sample compared with vol. obtained from 0.1 g $NaNO_3$, both measured under identical conditions.

After measuring tube has been removed, quickly insert over delivery tube another tube filled with soln of commercial NaOH (1+1) and boil 1 min. longer to assure absence of NO. Add another 50 ml portion std soln into app. and repeat detn. Then run sample in same manner, making certain to expel all NO and rinsing both casserole and separator 3 or 4 times; 6 to 8 detns may be made, excluding 2 stds. Finally run another std. The 3 stds should check within 0.5 ml on 30–35 ml; 0.1 g $NaNO_3$ should give 26.36 ml NO at 0° and 760 mm pressure. Report results as % $NaNO_3$.

Xylenol Method (5)—First Action

23.013 APPARATUS

Use simple distn app., including distn bulb. Type of glass condenser utilizing thin, rapidly moving H_2O film as cooling medium (West type) is recommended. Quickly remove any nitroxylenol solidifying in condenser by stopping H_2O flow and letting condenser become warm.

23.014 REAGENTS

(a) *m-Xylenol.*—1-hydroxy-2,4-dimethylbenzene. Eastman No. 1150, or equiv.

(b) *Silver ammonium hydroxide soln.*—Dissolve 5 g *nitrate-free* Ag_2SO_4 in 60 ml NH_4OH. Heat to boiling, conc. to ca 30 ml, cool, and dil. to 100 ml with H_2O.

(c) *Bromocresol green indicator.*—Dissolve 0.1 g bromocresol green in 1.5 ml 0.1*N* NaOH, and dil. to 100 ml with H_2O.

(d) *Nitrate std soln.*—Dissolve 0.1804 g recrystd KNO_3 in H_2O and dil. to 1 L, or dil. 17.85 ml 0.1*N* HNO_3 to 1 L. 10 ml contains 0.25 mg nitrate N.

23.015 DETERMINATION

Mix 5–10 g finely comminuted and thoroly mixed sample with 80 ml warm H_2O. Break up all lumps and heat on steam bath 1 hr, stirring occasionally. Transfer to 100 ml vol. flask, cool, dil. to vol., and mix. Filter, or let settle, and pipet 40 ml filtrate, or supernatant, into 50 ml vol. flask. (No correction for vol. occupied by meat is necessary.) Add 3 drops bromocresol green indicator. Add H_2SO_4 (1+10) dropwise until color changes to yellow. Oxidize nitrites to nitrates by adding 0.2*N* $KMnO_4$ soln dropwise with shaking until faint pink remains ca 1 min. Add 1 ml H_2SO_4 (1+10) and 1 ml *phosphotungstic acid soln* (20 g/100 ml). Dil. to mark, mix, and filter.

Measure into 500 ml flask (erlenmeyer is satisfactory) aliquot (not >20 ml) contg 0.025–0.25 mg nitrate N. (If >20 ml is required, make

slightly alk. and conc. by evapn.) Add enough Ag-NH_4OH soln to ppt all chlorides and most of excess phosphotungstic acid. (Slight excess of the Ag reagent is not harmful; 1 or 2 ml is usually enough.) Without decanting or filtering, add vol. H_2SO_4 (3+1) ca 3 times vol. liquid in flask. Stopper flask, mix, cool to ca 35°, add 0.05 ml (1–2 drops) of the *m*-xylenol, stopper, shake, and hold 30 min. at 30–40°.

(Yellow to brownish yellow color, indicative of nitrates, appears. Bright red ppt, due to incomplete removal of phosphotungstic acid, may also appear. Slight excess of phosphotungstic acid causes no interference but large excess may do so.)

After nitration is complete, add 150 ml H_2O, taking care to wash off stopper, and distill 40–50 ml into receiver contg 5 ml NaOH (10 g/L). Transfer distillate to 100 ml vol. flask, dil. to vol. with H_2O, and det. nitrate N by comparing reading of color of suitable aliquot with std curve.

Prep. color std from 10 ml of the nitrate std as directed previously, using 0.05 ml of the *m*-xylenol and 30 ml H_2SO_4 (3+1), and dilg distillate to 500 ml.

Nitrites (*6*)—First Action

(Applicable to cured meats)

23.016 REAGENTS

(a) *Modified Griess reagent.*—Dissolve 0.5 g sulfanilic acid in 150 ml 15% (by vol.) HOAc. Boil 0.1 g α-naphthylamine or 0.125 g of the hydrochloride in 20 ml H_2O until dissolved and pour while hot into 150 ml of the dil. HOAc. Mix the 2 solns, filter if necessary, and store in brown glass bottle.

(b) *Nitrite std soln.*—Dissolve 1.1 g $AgNO_2$, **13.042**(c), in nitrite-free H_2O, ppt the Ag with NaCl soln, dil. to 1 L, mix, and let settle. Dil. 100 ml to 1 L, and then 10 ml of this soln to 1 L, using in each case nitrite-free H_2O. 1 ml final soln =0.0001 mg N.

23.017 DETERMINATION

Weigh 5 g finely comminuted and thoroly mixed sample into 50 ml beaker. Add ca 40 ml nitrite-free H_2O heated to 80°. Mix thoroly with glass rod, taking care to break up all lumps, and transfer to 500 ml vol. flask. Wash beaker and rod thoroly with successive portions of the hot H_2O, adding all washings to flask. Add enough hot H_2O to bring vol. to ca 300 ml, transfer flask to steam bath, and let stand 2 hr, shaking occasionally. Add 5 ml satd $HgCl_2$ soln and mix. Cool to room temp., dil. to mark with nitrite-free H_2O, and mix again. Filter, dil. suitable aliquot to mark in 50 ml vol. flask, add 2 ml reagent, mix, and let color develop 1 hr. Transfer suitable portion of soln to photometer cell and det. absorbance at wavelength of 520 mμ, setting instrument to zero absorbance with blank of 50 ml H_2O plus 2 ml reagent.

Det. nitrite present by comparison with std curve prepd as follows: Dil. suitable vols std nitrite soln to mark in 50 ml vol. flasks, add 2 ml reagent, and proceed as above. Std curve is straight line to 5 mmg N in final soln.

Creatin—Official

23.018 PREPARATION OF SOLUTION

Exhaust 7–25 g sample (depending upon H_2O content) as follows: Weigh into 150 ml beaker, add 5–10 ml cold (15°) NH_3-free H_2O, and stir to homogeneous paste. Add 50 ml cold H_2O, stir at 3 min. intervals during 15 min., let stand 2–3 min., and decant liquid thru quant. filter, collecting filtrate in 500 ml vol. flask. Drain beaker, pressing out liquid from meat residue with glass rod. Add to residue in beaker 50 ml cold H_2O, stir 5 min., let stand 2–3 min., and decant as before. If much meat is transferred to filter, return it to beaker with glass rod. Repeat extns, using two 50 ml portions and four 25 ml portions cold H_2O. After last extn transfer entire insol. portion to filter and wash with three 10 ml portions H_2O, letting material drain thoroly after each addn. Dil. to mark and mix thoroly.

Measure 150 ml ext. into 250 ml beaker and evap. to 40 ml on steam bath, stirring occasionally. Neutralize to phthln, using indicator outside the soln. Add 1 ml 0.1*N* HOAc and boil gently 5 min. (Coagulum should sep. at once, leaving clear liquid.) Filter thru quant. paper, wash beaker thoroly 4 times with hot H_2O, wash coagulum on filter 3 times, and discard coagulum.

23.019 DETERMINATION

Evap. filtrate and washings, **23.018**, to 5–10 ml, transfer with min. quantity hot H_2O to 50 ml vol. flask, keeping vol. <30 ml, add 10 ml 2*N* HCl, and mix. Hydrolyze 20 min. in autoclave at 117–120°, let flask cool somewhat, and chill under running H_2O. Partially neutralize excess acid by adding 7.5 ml 10% NaOH soln (CO_2-free), dil. to mark, and mix.

Make preliminary reading after carrying thru reaction on 20 ml with Duboscq colorimeter to det. vol. needed to obtain reading of ca 8 mm. Transfer such vol. to 500 ml vol. flask and add 10 ml 10% NaOH soln and 30 ml *satd (1.2%) picric acid soln.* Mix, rotate 30 sec., and let stand exactly 4.5 min. Dil. to mark at once with H_2O, shake thoroly, and compare, preferably in Duboscq colorimeter, with std soln prepd by treating with NaOH and picric acid, and dilg to 500 ml as above, 50 ml of soln contg 1.603 g *creatinin Zn chloride* in 1 L 0.1*N* HCl (1 ml=0.001 g creatinin; g creatinin $\times$1.16 =g creatin).

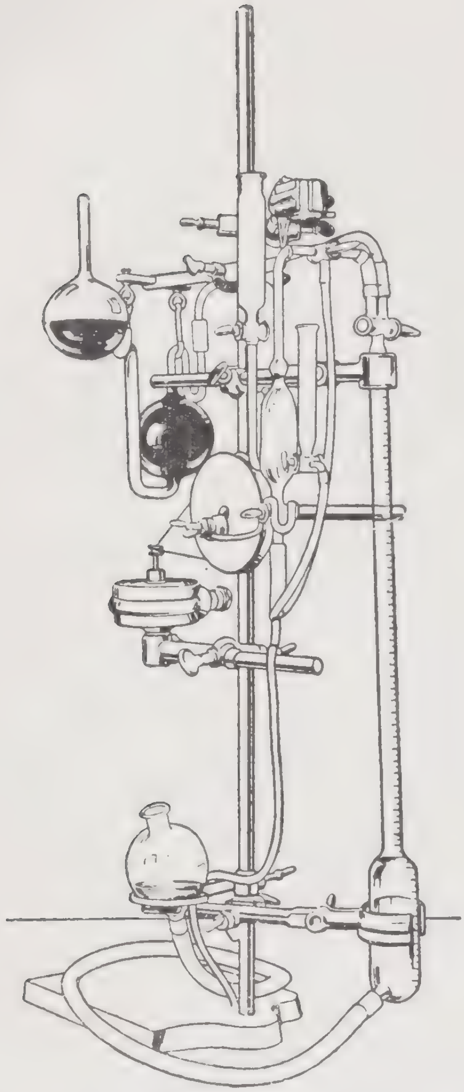

FIG. 41.—VAN SLYKE APPARATUS FOR DETERMINATION OF AMINO NITROGEN

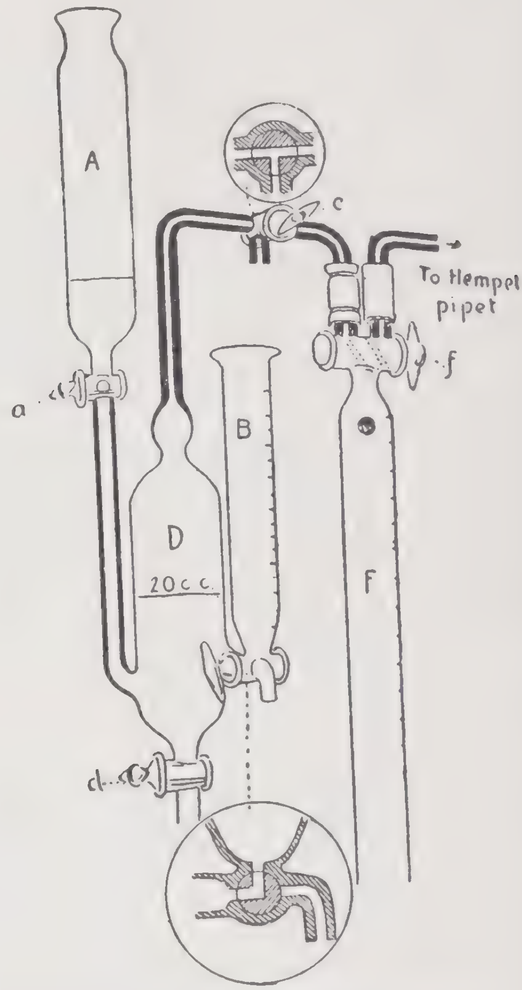

FIG. 42.—DETAILS OF DEAMINIZING BULB AND CONNECTION

Amino Nitrogen

Van Slyke Method (7)—First Action

23.020 APPARATUS

Use app. shown in Figs. 41 and 42, former illustrating manner in which entire app. is arranged and latter showing details of deaminizing bulb and connections. The Hempel gas pipet is filled with soln contg 50 g $KMnO_4$ and 25 g KOH/L.

23.021 DETERMINATION

Fill, with H_2O, buret (*F*), capillary tube leading to Hempel pipet, and other capillary as far as *c*. Introduce into *A* enough HOAc to fill $\frac{1}{5}$ of *D*, etching tube *A* with mark to measure this quantity. Let acid run into *D*, and turn cock *c* to let air escape from *D*. Pour $NaNO_2$ soln (300 g/L) into *A* until *D* is filled and enough excess is present to rise little above cock into *A*. *A* is also marked for measuring off this quantity. Close gas exit from *D* at *c*, and with *a* open, shake *D* few sec. until liquid is forced down to 20 ml mark in *D*. Close *a*, open *c*, and shake app. rapidly with motor 2 min. (These operations are for purpose of expelling all air from *D*.) Turn *c* and *f* so that *D* and *F* are connected.

Measure in *B* 10 ml or less, as case may be, of sample soln contg not >20 mg amino N (1–2 g sample in case of meat exts) and let it run into *D*. Connect *D* with motor as in Fig. 41 and shake 5 min.

If soln of sample is viscous and threatens to foam over, rinse out *B*, and thru it introduce little *capryl alcohol* into *D*, or if it is known beforehand that sample will cause excessive foaming, intro-

duce little capryl alcohol into D thru B, rinsing B with alcohol and ether or drying with roll of filter paper before adding soln of sample.

During shaking, N mixed with NO is evolved, the gases being collected in F. Force all gas in D into F, by opening a and filling D with liquid from A. Connect F with Hempel pipet and force gas into latter by means of leveling bulb, letting cock a remain open during this and succeeding operation to permit displacement of liquid in D by NO formed in interval. Connect driving rod with pipet by lifting hook from shoulder of D and placing other hook on opposite side of driving rod, over horizontal lower tube of pipet. Shaking pipet rather slowly for few min. completes absorption of NO except with almost completely exhausted $KMnO_4$ solns. Return gas to buret and adjust level with leveling bulb; note vol. N, temp., and barometric pressure, and calc. vol. N under std conditions of temp. and pressure. Obtain corresponding wt N, divide by 2, and from quotient calc. apparent % amino N in sample. Correct result for blank test performed as above, using 10 ml H_2O instead of sample soln. Quantity of gas obtained in blank is usually 0.3–0.4 ml, and nitrite solns giving much larger correction should be rejected.

With beef exts and similar prepns, 5 min. is enough to allow for completion of reaction in D. In general, same time serves for decomposition of α-amino acids, but with NH_3, methylamine, and most amines other than α-amines allow 1–1.5 hr. For detns on such substances mix sample soln with reagents, as described previously, let mixt. stand in app. till end of required time, and conclude reaction by shaking app. with motor 2–3 min. Continue detn as directed previously.

23.022 *Sørensen Method* (*8*)—*First Action*

To 20 ml filtrate, **23.018**, second paragraph, neutralized to phthln with $Ba(OH)_2$ or NaOH, or to 20 ml of equiv. ext. of the meat (sometimes larger vol. may be necessary) add 10 ml freshly prepd *phthln-formol mixt.* (50 ml 40% HCHO soln contg 1 ml 0.5% phthln soln in 50% alcohol, exactly neutralized with $0.2N$ $Ba(OH)_2$ or NaOH). Titr. mixt. with $0.2N$ $Ba(OH)_2$ until distinct red appears, add small but known excess $0.2N$ $Ba(OH)_2$, and back-titr. to neutrality with $0.2N$ HCl.

Conduct blank titrn with same reagents, using 20 ml H_2O in place of soln to be tested. From quantity $0.2N$ $Ba(OH)_2$ required to neutralize mixt., corrected for quantity used in blank titrn, calc. quantity amino N present (including NH_3 if this has not been removed). 1 ml $0.2N$ $Ba(OH)_2$ soln = 2.8 mg amino N.

23.023 Starchy Flour—Qualitative Tests—Procedure

(In chopped meat, sausage, deviled meat, etc.)

(**a**) Treat 5–6 g sample with boiling H_2O 2–3 min., cool mixt., and test supernatant with I soln, **28.025**(**d**). (In interpreting this test note that small quantity of starch may be present as result of use of spices. If strong reaction is given, cereal products are present. This qual. test may be replaced by microscopic examination, which discloses not only presence of added starch but also variety used.)

(**b**) *Not applicable in presence of cellulosic material other than that from starchy flour and spice.*—To 10 g sample in 100 ml graduated oil tube (ASTM conical form with stem graduated from 0 to 3 ml in 0.1 ml) add 50 ml 8% alc. KOH soln and digest on steam bath 1 hr, stirring occasionally. Dil. to 100 ml with alcohol, mix, and let stand 1 hr, rotating gently once or twice during this period to loosen particles on sides of tube. After 1 hr read vol. of sediment in tube. Vol. >1 ml, if sample contains spices, or >0.5 ml if only spice oils are present, indicates presence of added starchy flour. Vol. <3 ml indicates that <3% flour is present, and vol. >3.5 ml indicates presence of >3.5% flour. (If sample contains dried skim milk or dried corn sirup, before proceeding with test remove lactose or maltose by shaking 10 g in 100 ml centrifuge tube with two 50 ml portions warm H_2O and centrifuging and decanting after each shaking.)

23.024 Soybean Flour—Qualitative Test (*9*)**—Procedure**

Mix 10 g finely divided sample in 250 ml beaker with 75 ml 8% alc. KOH soln, and heat on steam bath until all meat is dissolved (30–45 min.). Transfer liquid and residue to 100 ml graduated sedimentation tube, dil. to 100 ml with alcohol, and let settle. Decant supernatant as completely as possible, and cover residue with ca 50 ml warm H_2O. Stopper tube and shake vigorously; let stand few min. until foam subsides; then transfer to 50 ml centrifuge tube, and centrifuge. Pour off and discard supernatant, and add 10 ml HCl to centrifuge tube. Stopper and shake, or mix contents thoroly with glass rod. Add ca 15 ml 25% alcohol, mix, and centrifuge. Pour off supernatant and examine residue under microscope for characteristic "hour-glass" or I-shaped cells (sometimes called "bearer cells"), preferably with polarized light.

23.025 Preservatives—Official— *See* Chap. **27**.

Qualitative Test for Agar—Official

23.026 REAGENTS

(a) *Trichloroacetic acid soln.*—25 g acid in 50 ml H_2O.

(b) *Iodine soln.*—Approx. 0.033N.

(c) *Benedict qualitative soln.*—*See* **15.155**(a).

23.027 PREPARATION OF SAMPLE

(a) *Boned chicken or meat.*—Refrigerate overnight to jell broth. With thin-blade spatula, sep. as much jell as possible, and warm on steam bath until it liquefies completely.

(b) *Consommé or broth.*—No prepn necessary.

23.028 DETECTION OF GUM

Transfer up to 40 ml liquefied jell from meat, or 40 ml consommé, to 100 ml beaker. Add 5 ml of the trichloroacetic acid soln, stir, and let stand 15–30 min. Transfer to 50 ml conical centrifuge tube and centrifuge 15–20 min. at ca 1200 rpm. Decant clear supernatant into 250 ml (8 oz) centrifuge bottle or nursing bottle, add 4–5 vols alcohol, and let stand until ppt coagulates, or overnight. (No ppt indicates absence of gums.) Centrifuge at 1200 rpm 15–30 min. until ppt packs to bottom of centrifuge bottle. Carefully decant the alcohol, taking care not to disturb packed gum ppt. Remove few remaining drops of alcohol by spontaneous drying or by gentle air current. Add 1 drop of the 0.033N I soln. Evanescent violet or black color indicates presence of agar. (Negative test does not necessarily mean agar is absent.)

Add 3 ml hot H_2O and warm on steam bath until gum ppt dissolves. Chill gum soln in ice and H_2O mixt. Thickening, or stiff jell, indicates agar. Warm cooled mixt. on steam bath, transfer to 50 ml beaker, rinse centrifuge bottle with 3–4 ml H_2O, and add rinsings to jell soln. Add 1 ml HCl and boil 30 sec. Transfer 1 ml hydrolyzed gum soln to test tube, neutralize with 10% NaOH soln, using litmus paper as indicator (ca 2 ml required), remove litmus paper, add 5 ml of the Benedict soln, and boil cautiously over free flame 30–60 sec. Green, yellow, or brick-colored ppt after spontaneous cooling indicates agar (or other hydrolyzable gum).

23.029 Nonfat Dry Milk (Qualitative Test) (*10*)—Procedure

(In absence of maltose)

To 10 g comminuted sample in small beaker add 20 ml hot (70–90°) H_2O. Mix thoroly and filter. Transfer 4 ml filtrate to test tube, add 3–4 drops *5% methylamine hydrochloride soln*, and boil 30 sec. Remove from flame, add 3–5 drops 20% NaOH soln, and shake 10 sec. Yellow color appears immediately, which slowly changes to carmine if lactose is present, indicating presence of nonfat dry milk.

Lactose (*11*)—First Action

23.030 REAGENTS

(a) *Acclimated yeast suspension (for use in presence or absence of maltose).*—Macerate 2 cakes (0.6 oz each) bakers' yeast and wash with 3 ca 50 ml portions H_2O, centrifuging between washings. Prep. medium contg 1.0 g anhyd. $MgSO_4$, 2.0 g NH_4Cl, 1.0 g anhyd. K_2HPO_4, 0.5 g KCl, 0.02 g $FeSO_4.7H_2O$, 0.7 g peptone, and 20.0 g tech maltose. Dissolve each ingredient in small amount H_2O and add, in order given, to flask contg ca 500 ml H_2O. Dil. to 1 L. Warm, filter, bring filtrate to rolling boil, and let cool to room temp. Add washed yeast to 1 L medium and incubate ca 24 hr at 30°, stirring frequently first few hr. Sep. yeast by decanting and centrifuging, wash twice with H_2O, add to 1 L fresh medium, and incubate addnl 24 hr with agitation first few hr. Sep. yeast from medium, wash thoroly at least 4 times with H_2O, dil. to 100 ml, and refrigerate. Yeast remains active 2–3 weeks. (Yeast may remain active longer if frozen.)

(b) *Washed yeast suspension (for use in absence of maltose).*—Mix 2 cakes bakers' yeast to smooth suspension with ca 150 ml H_2O. Centrifuge 5 min. and discard aq. layer. Repeat mixing with H_2O and centrifuging 4 more times, or until supernatant after centrifuging is practically clear. Again suspend the yeast in H_2O and dil. with H_2O to 100 ml. Store in refrigerator at ca 4° and shake well before using. Discard after 2 weeks.

(c) *Benedict soln.*—Dissolve 16 g $CuSO_4.5H_2O$ in 125–150 ml H_2O. Dissolve 150 g Na citrate ($Na_3C_6H_5O_7.2H_2O$), 130 g anhyd. Na_2CO_3, and 10 g $NaHCO_3$ in ca 650 ml hot H_2O. Combine the 2 solns, cool, dil. to 1 L, and filter.

(d) *Lactose std soln.*—Dissolve 1.5789 g lactose $.H_2O$ in H_2O and dil. to 1 L (1 ml = 1.5 mg anhyd. lactose).

(e) *Iodine std soln.*—Mix 5.08 g I with 10.2 g KI, dissolve in small quantity of H_2O, filter, and dil. to 1 L.

(f) *Sodium thiosulfate std soln.*—Dissolve 9.92 g $Na_2S_2O_3.5H_2O$ in H_2O and dil. to 1 L.

(g) *Dilute acetic acid.*—Dil. 240 ml HOAc to 1 L with H_2O.

(h) *Dilute phosphoric acid.*—Dil. 240 ml H_3PO_4 to 1 L with H_2O.

(i) *Citric acid-phosphate buffer.*—pH 4.8. Mix solns in proportions of 10.14 ml 0.1M citric acid (19.21 g/L) and 9.86 ml 0.2M Na_2HPO_4 (28.4 g anhyd./L), and adjust to pH 4.8, using pH meter.

Store in refrigerator and discard if soln becomes turbid.

23.031 DETERMINATION (IN PRESENCE OF MALTOSE)

Place 10 g sample in 100 ml vol. sugar flask, add small amount of H_2O, and break up sample by agitation. Add ca 50 ml H_2O and warm on steam bath ca 30 min. Cool to room temp., add 2 ml HCl, and dil. to vol., using bottom of fat layer as meniscus. Add 5.0 ml *20% phosphotungstic acid soln*, mix well, let stand few min., and filter thru moist paper. Pipet 40 ml filtrate into 50 ml vol. flask and neutralize just to acid side of chlorophenol red or other indicator which shows pH change at ca 4.8. Add 5 ml of the buffer soln, dil. to vol., and mix.

Transfer ca 40 ml of this soln to centrifuge tube to which 5 ml yeast suspension, **23.030(a)**, has been added and from which H_2O has been sepd. Mix yeast and sample well and incubate 3 hr at 30°, stirring frequently. Centrifuge and det. reducing sugars.

Pipet 10 ml clear soln into 300 ml erlenmeyer, add 20 ml Benedict soln, **23.030(c)**, bring to boil in 3–5 min., and boil slowly exactly 3 min. Remove from heat, cool, and add 100 ml H_2O and 10 ml dil. HOAc, **23.030(g)**, slowly while swirling. Add ca 30% excess std I, **23.030(e)** (15 ml for ca 1.5% lactose), and agitate to dissolve the CuO. Let flask stand at least 5 min., add 20 ml dil. H_3PO_4, **23.030(h)**, and titr. excess I with std $Na_2S_2O_3$ soln, **23.030(f)**, using starch indicator, **29.046(d)**.

Det. lactose: I ratio by using 10 ml std lactose soln and carrying thru detn as above, beginning "... add 20 ml Benedict soln, ..." Det. I: $Na_2S_2O_3$ ratio by using 10 ml H_2O and carrying thru detn as above.

% lactose $= 100\ KV/A$, where $K =$ g lactose/ml I soln, $V =$ vol. I soln consumed, and $A =$ g sample in aliquot, considering vol. original sample soln as 100 ml rather than 105 ml, to correct for vol. occupied by meat.

23.032 DETERMINATION (IN ABSENCE OF MALTOSE)

Prep. soln as in **23.031**, first par. Place 5 ml washed yeast suspension, **23.030(a)** or **(b)**, in lipless centrifuge tube, centrifuge, and drain and discard supernatant. Add 40 ml prepd soln to yeast residue in centrifuge tube, stopper, and shake vigorously to dislodge and suspend yeast. Let stand with occasional shaking 1 hr; then centrifuge and det. lactose in the clear soln as in **23.031**, third par.

Starch (*12*)—Official

(Not applicable to liver products)

23.033 REAGENTS

(**a**) *Zinc acetate soln.*—Dissolve 12 g $Zn(OAc)_2.2H_2O$ in H_2O and dil. to 100 ml.

(**b**) *Potassium ferrocyanide soln.*—Dissolve 6 g $K_4Fe(CN)_6.3H_2O$ in H_2O and dil. to 100 ml.

(**c**) *Copper sulfate soln.*—Dissolve 40.0 g $CuSO_4.5H_2O$ in H_2O and dil. to 1 L.

(**d**) *Alkaline tartrate soln.*—Dissolve 200 g Rochelle salt and 150 g NaOH in hot H_2O, filter, and dil. to 1 L.

(**e**) *Dextrose std soln.*—Dissolve 0.40 g pure dextrose in H_2O and dil. to 200 ml.

(**f**) *Starch indicator soln.*—Mix 1 g powd. sol. starch with 20 ml cold H_2O. Pour mixt. into 500 ml boiling H_2O and boil 10 min. Cool, and add few drops $CHCl_3$.

(**g**) *Phosphotungstic acid soln.*—Dissolve 20 g phosphotungstic acid in H_2O, dil. to 100 ml, and filter.

23.034 EXTRACTION AND HYDROLYSIS

Weigh 10 g finely ground and thoroly mixed sample into 250 ml heat-resistant centrifuge bottle. If fat content is sufficiently high as to interfere with subsequent filtering, add 25 ml petr. ether, mix thoroly with glass rod, decant, and repeat with 2 addnl 25 ml portions petr. ether. Add 100 ml H_2O, 5 ml freshly prepd $Zn(OAc)_2$ soln, and 5 ml freshly prepd $K_4Fe(CN)_6$ soln. Stopper tightly and let stand 15 min., shaking vigorously several times during this period. Centrifuge 15 min. at 1500 rpm. Decant supernatant into 12.5 cm Whatman No. 3 filter paper in conical funnel, using light suction. To residue in centrifuge bottle add 25 ml freshly prepd soln contg 1 ml of the $Zn(OAc)_2$ plus 1 ml of the $K_4Fe(CN)_6$ solns/200 ml soln. Let stand 10 min., shaking several times during this period; then centrifuge 10 min. at 1500 rpm and decant thru same paper. Repeat last extn with addnl 25 ml $Zn(OAc)_2$-$K_4Fe(CN)_6$ washing soln. Rinse stopper with H_2O.

Transfer funnel contg filter paper to centrifuge bottle. From graduated cylinder contg 90 ml hot 1.5*N* HCl (ca 70°) pour 40 ml into paper to melt adhering fat and to free starch. Poke hole in tip of paper and let acid run into centrifuge bottle. Wash paper with remainder of acid soln. Suspend bottle in open boiling H_2O bath so that level of H_2O in bath is at approx. level of soln within bottle. Do not reflux. Hydrolyze exactly 1.5 hr, keeping H_2O level of bath at original position, stirring contents of bottle occasionally. Do not

transfer paper to centrifuge bottle, as it will hydrolyze and give high values.

Cool immediately. (If necessary, sample may stand overnight at this point.) Make just alk. to litmus with 20% NaOH (ca 27 ml) and then add 10 ml HCl (1+2). Transfer to 200 ml phosphoric acid flask or 200 ml erlenmeyer marked at 200 ml. Rinse centrifuge bottle with 15 ml phosphotungstic acid soln, followed by several 10 ml portions H_2O. Dil. to vol., with fat layer, if any, just above mark. Stopper, shake, let stand ca 30 min., and filter soln thru Whatman No. 1 paper.

23.035 DETERMINATION OF REDUCING SUGARS

Pipet 20 ml filtrate into heat-resistant 200 ml erlenmeyer. Pipet in 20 ml of the $CuSO_4$ soln and 20 ml of the alk. Rochelle salt soln. Bring to boil within 2 min., swirling occasionally, and continue boiling 1 min. Cool immediately under running H_2O, transfer to 200 ml vol. flask, dil. to vol. with H_2O, stopper, and shake.

Pipet 50 ml soln into 200 ml erlenmeyer. Add 25 ml 10% KI and 5 ml H_2SO_4 (1+3). Titr. with ca 0.025N $Na_2S_2O_3$ soln, adding 2 ml starch indicator and ca 2.0 g solid KSCN when yellow color has almost disappeared. (1 drop $Na_2S_2O_3$ soln should change color from blue to white or faint lilac shade.) Det. blank, using 20 ml H_2O instead of filtrate, starting at first par. Conduct detn on 20 ml std dextrose soln similarly. % Starch $=4\times0.9\times(A-B)/(A-C)$, where A = blank titrn in ml; B = sample titrn in ml; C = std dextrose titrn in ml; 0.9 = factor to convert dextrose to starch.

MEAT EXTRACTS AND SIMILAR PRODUCTS

23.036 Preparation of Sample—Procedure

Remove liquid and semi-liquid meat exts and similar prepns from container and mix thoroly. (Slight heating expedites mixing of pasty exts.) Carefully remove from bottom of container sediment that forms in many liquid prepns and include in sample. If sample is in form of cubes, grind 10–12 cubes in mortar.

23.037 Moisture—Official

Proceed as in **22.003**, using ca 2 g powd. prepns, ca 3 g pasty prepns, and 5–10 g liquid exts, according to solid content. Dry powd. prepns directly without admixture. Dissolve pasty prepns in H_2O and dry with enough ignited sand, asbestos, or pumice stone to absorb soln. When glycerol is present, proceed as in **22.007**.

23.038 Ash—Official

Proceed as in **29.012** or **29.013**. Add enough H_2O to pasty prepns to effect soln and evap. to dryness so as to distribute solids evenly over bottom of dish.

23.039 Total Phosphorus—Official

Destroy org. matter as in **2.018(c)** or **(d)**, and proceed as in **2.019** or **2.022**.

23.040 Chlorides—Official

Dissolve ca 1 g prepd sample, **23.036**, in 20 ml 5% Na_2CO_3 soln and proceed as in **6.065–6.066**.

23.041 Total Nitrogen—Official—*See* 2.036

23.042 Creatin—Official

Dissolve ca 7 g sample in cool (20°) NH_3-free H_2O in 150 ml beaker, transfer soln to 250 ml vol. flask, dil. to mark, and mix thoroly. Transfer 20 ml aliquot to 50 ml vol. flask and proceed as in **23.019**. Subtract from total creatinin value equiv. of preformed creatinin, **23.043**, and multiply difference by 1.16 to convert to creatin. Express result as % creatin.

23.043 Creatinin—Official

Measure ca 5 ml soln used in **23.042** into 500 ml vol. flask, add 10 ml 10% NaOH soln and 30 ml *satd (1.2%) picric acid soln*, mix, and rotate 30 sec. Let stand exactly 4.5 min. and dil. to mark at once with H_2O. Shake thoroly and read color in colorimeter after standing. If reading is <7 or >9.5 mm, repeat, calcg quantity of soln necessary to obtain reading of ca 8 mm. Express result as % creatinin, making calcns as in **23.019**.

23.044 Preservatives—Official—*See* Chap. 27

23.045 Sulfur Dioxide (Distillation Method)—*See* 27.078

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24. Metals, Other Elements, and Residues in Foods

INORGANIC RESIDUES

ARSENIC

Gutzeit Method (1)—Official

24.001 REAGENTS

(a) *Stannous chloride soln.*—Dissolve 40 g As-free $SnCl_2.2H_2O$ in HCl and dil. to 100 ml with HCl.

(b) *Zinc.*—Use 20- or 30-mesh, As-free granulated Zn (needs no preliminary treatment), or As-free stick Zn either cut into equal 1 cm lengths, or melted and cast into pellets in porcelain mold drilled (for example) 9 mm diam. and 12.5 mm deep. Activate the pieces of Zn with HCl (1+3), to which has been added 2 ml of the $SnCl_2$ soln, letting action continue 15 min. Discard distinctly inactive or overactive pieces and pour off liquid. Wash Zn free from acid with clear tap H_2O, and rinse with hot H_2O. Select uniformly etched non-pitted Zn and store in suitable receptacle. To maintain supply of uniform Zn adopt system of rotation by withdrawing Zn from original receptacle until stock is exhausted and storing used Zn in second receptacle after discarding non-uniform or deeply pitted pieces. Draw Zn from second receptacle after washing it with clear running H_2O. Repeat procedure until pieces are too small for further use.

(c) *Potassium iodide soln.*—Dissolve 15 g KI in H_2O and dil. to 100 ml.

(d) *Sand.*—Clean 30-mesh (thru No. 30 but not No. 40) white sea sand by washing successively with hot 10% NaOH soln, hot HNO_3, and hot H_2O. Dry the clean sand.

(e) *Mercuric bromide paper.*—Use commercial As papers cut from paper of uniform wt and texture into strips exactly 2.5 mm wide and ca 12 cm long. (Uniformity in width and texture of paper is of great importance in this comparison method. Irregular texture produces irregular impregnation with consequent inaccurate results.) To sensitize, soak strips in 3–6% (optimum 5%) filtered soln of $HgBr_2$ in alcohol, 1 hr or longer according to quantity, character, and activity of Zn used. (Attenuated, unsatisfactory stains, caused by over-rapid evolution of AsH_3, can be shortened and intensified by increasing concn of $HgBr_2$ and vice versa.) If strips are in sheets, cut off 2 sides before soaking and leave strips attached at ends. After sensitization remove strips and dry individual ones on glass rods; dry groups by waving them in air. Place strips when nearly dry between clean sheets of paper and subject to pressure long enough to take out bends or curls. Store in dry, dark place. (Aging of impregnated strips usually results in markedly fainter and longer stains. Desirable types of stain result from use of impregnated strips not >2 days old.) When ready for use, cut individual strips off squarely 0.5″ from one end and insert this end into narrow tube of app. Handle sheets by paper attached to either end and cut in half just before use. Strips must be clean and free of any contamination.

(f) *Arsenic std soln.*—Dissolve 1 g As_2O_3 in 25 ml 20% NaOH soln. Sat. soln with CO_2 and dil. to 1 L with recently boiled H_2O (1 ml = 1 mg As_2O_3). Dil. 40 ml of this soln to 1 L. Dil. 50 ml of the dild soln to 1 L and use to prep. std stains (1 ml = 0.002 mg As_2O_3). Soln contg 0.001 mg As_2O_3 may also be prepd if desired. Prep. fresh dil. solns frequently.

24.002 APPARATUS

(a) *Generators and absorption tubes.*—*See* Fig. 43. Use 2 oz wide-mouth bottles of uniform capacity and design as generators, and fit each by

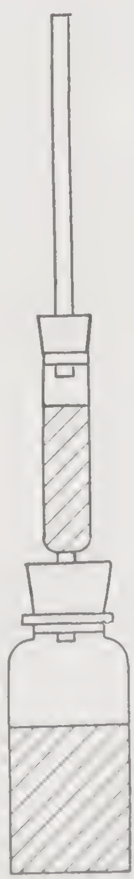

FIG. 43.—GENERATOR USED WITH GUTZEIT METHOD FOR DETERMINATION OF ARSENIC

means of perforated stopper with glass tube 1 cm diam. and 6–7 cm long, with addnl constricted end to facilitate connection. Place small wad of glass wool in constricted bottom end of tube and add 3.5–4 g of the sand, taking care to have same quantity in each tube. Moisten sand with 10% $Pb(OAc)_2$ soln and remove excess by light suction. Clean sand when necessary by treatment (do not remove sand from tube) with HNO_3 followed by H_2O rinse and suction. Treat with the $Pb(OAc)_2$ soln. If sand has dried thru disuse, clean and remoisten it as directed. Connect tube by means of rubber stopper with narrow glass tube 2.6–2.7 mm i.d. and 10–12 cm long, and introduce clean end of the strip of $HgBr_2$ paper. (3 mm bore allows strip to curl, which results in uneven stain and poor end point.) Clean and dry tube before inserting $HgBr_2$ paper. (Ordinary pipe cleaner may be used.)

(**b**) *Water bath.*—Use constant temp. H_2O bath. If no H_2O bath is available, use flat-bottom container of suitable depth and capacity. (Deep H_2O bath is suggested to insure uniform conditions during evolution and absorption of the AsH_3.)

24.003 PREPARATION OF SAMPLE

(For details of convenient churn-type washer that will remove arsenical spray residues from firm fruits or vegetables with an aq. NH_4NO_3-HNO_3 soln *see* Fahey, Cassil, and Rusk (*2*). Digest aliquot of the "strip" soln and proceed as in (**a**).)

(**a**) *For fresh fruits* (*apples, pears, or similar products*).—Weigh and peel representative sample of fruit (1–5 lb). At blossom and stem ends cut out all flesh thought to be contaminated with As compounds and include with peelings, if desired. Place peelings in 1 or more 800 ml Pyrex Kjeldahl flasks. (As-free Pyrex glassware and "wet ashing" app. of Duriron are available.) Add 25–50 ml HNO_3; then add cautiously 20 ml H_2SO_4. Place each flask on asbestos mat with 2″ hole. Warm slightly and discontinue heating if foaming becomes excessive.

When reaction has quieted, heat cautiously and rotate flask occasionally to prevent caking of sample upon glass exposed to flame. Maintain oxidizing conditions in flask at all times during digestion by cautiously adding small quantities of HNO_3 whenever mixt. turns brown or darkens. Continue digestion until org. matter is destroyed and SO_3 fumes are copiously evolved. (Final soln should be colorless, or at most light straw color.) Cool slightly, and add 75 ml H_2O and 25 ml satd NH_4 oxalate soln to assist in expelling oxides of N from soln. Evap. again to point where fumes of SO_3 appear in neck of flask. Cool, and dil. with H_2O to 500 or 1000 ml in vol. flask.

(**b**) *For dried fruit products.*—Prep. sample by alternately grinding and mixing 4–5 times in food chopper. Place 35–70 g portions in 800 ml Kjeldahl flasks, and add 10–25 ml H_2O, 25–50 ml HNO_3, and 20 ml H_2SO_4. Continue digestion as in (**a**). Dil. digested soln to 250 ml.

(**c**) *For small fruits, vegetables, etc.*—Use 70–140 g sample and digest as in (**a**) or (**b**).

(**d**) *For materials other than* (**a**), (**b**), *or* (**c**).—Digest 5–50 g, according to moisture content and quantity of As expected, as in (**a**) or (**b**). Dil. to definite vol. dictated by circumstances.

(**e**) *For products containing stable organic As compounds, products liable to yield incompletely oxidized organic derivatives that inhibit arsine evolution, or products that are otherwise especially difficult to digest.*—Shrimp, tobacco, oils, and sometimes other products require special treatment to complete oxidation of org. As to inorg. As_2O_5, or to destroy org. interferences previous to As detn. For details consult following references:

(1) C. R. Gross, *Ind. Eng. Chem., Anal. Ed.* **5**, 58(1933).

(2) Carey, Blodgett, and Satterlee, *Ibid.* **6**, 327 (1934).

(3) Remington, Coulson, and von Kolnitz, *Ibid.*, 280.

(4) C. C. Cassil, *J. Assoc. Offic. Agr. Chemists* **20**, 171(1937).

Dil. the As solns obtained by these special methods of prepn to definite vol.

(**f**) *For ultra-micro quantities of As, very labile forms of As, and vacuum-accelerated Gutzeit reduction system for mercuric bromide spot filtration.*—Consult Satterlee and Blodgett, *Ind. Eng. Chem., Anal. Ed.* **16**, 400(1944).

24.004 ISOLATION OF ARSENIC

Before making detns isolate the As, when interfering substances are present in digests (*e.g.*, pyridine from tobacco), or when samples contain excessive quantities of salts, or H_2SO_4 from digestions. Consult (1) of **24.003**(**e**) for method of isolation of As after digestion, or isolate As by $AsCl_3$ distn as in bromate method, **24.009.** Gelatin may be hydrolyzed with HCl and As isolated as in (1) of **24.003**(**e**).

24.005 DETERMINATION

Det. the acid (HCl or H_2SO_4 according to previous treatment), by titrn if necessary, in definite vol. of sample soln. Place aliquots contg 0.01–0.03 mg As_2O_3 (0.020–0.025 mg is optimum), and not >30 ml, in Gutzeit generators. If As in aliquot taken is found to be outside limits specified, repeat with proper aliquot. If aliquot contains only HCl, add enough HCl to make total vol. of 5 ml HCl; if it contains H_2SO_4, add enough 25% As-free NaOH soln (keep in As-free Pyrex) to

exactly neutralize it and add 5 ml HCl, or add enough HCl to the H_2SO_4 in aliquot to make total vol. of 5 ml of the mixed acids. Cool when necessary and add 5 ml of the KI reagent and 4 drops of the $SnCl_2$ soln, **24.001**(a).

Prep. stds corresponding to 0.010, 0.020, and 0.030 mg As_2O_3 from As std soln, **24.001**(f). Since stds *must* contain same kind and quantities of acid as samples, add 5 ml HCl, or H_2SO_4 and HCl (total 5 ml), according to prior treatment of unknown. If the H_2SO_4 has been neutralized, add equiv. quantity of As-free Na_2SO_4 to stds. Mix, and let stand 30 min. at not $<25°$ or 5 min. at 90°. Dil. with H_2O to 40 ml.

Prep. generator as in **24.002** and center strip of $HgBr_2$ paper carefully in narrow tube. According to activity of the Zn, add to each std and sample 10–15 g activated stick Zn or 2–5 g granulated Zn and add same quantity to each generator. Equalize as far as possible surface area of Zn exposed in std and sample. If sheets of strips are used, prep. sample and std strips from same strip-group.

Immerse app. to within 1″ of top of narrow tube in H_2O bath kept at temp. of 20–25°, and let evolution proceed 1.5 hr. Remove strip, and average length of stains on both sides in mm. Plot graph of std strips on cross-section paper, using length in mm as ordinate and mg As_2O_3 as abscissa. (Prepn of std graph averages errors of individual stds. Reading strip from such graph is considered more convenient and accurate than comparing strips themselves.) Locate length of unknown strip on std graph and read off on abscissa quantity of As present. Report only to third decimal as grains As_2O_3/lb. Take smaller or larger aliquots when stain is longer or shorter than highest or lowest std, resp. (Grains/lb) $\times 143 =$ ppm; ppm $\times 0.007 =$ grain/lb.

Make blanks frequently. Blanks should not show >0.001 mg As_2O_3.

Bromate Method (3)—First Action

(For detn of As in plants and food products where sample of convenient size for digestion yields at least 0.005 grain (0.324 mg) of As_2O_3)

24.006 REAGENTS

(a) *Ammonium oxalate-urea soln.*—To satd aq. soln of NH_4 oxalate add 50 g urea/L.

(b) *Hydrazine sulfate-sodium bromide soln.*—Dissolve 20 g hydrazine sulfate and 20 g NaBr in 1 L HCl (1+4).

(c) *Sodium chloride.*—Commercial salt, uniodized.

(d) *Potassium bromate std soln.*—Dissolve 0.1824 g $KBrO_3$ in H_2O and dil. to 1 L. (1 ml = 0.005 grain As_2O_3). Stdze by titrn against std As_2O_3 soln, (e), making titrn at ca 90° after adding ca 100 ml H_2O and 25 ml HCl, in order to simulate conditions under which samples will be titrd. 1 ml of the $KBrO_3$ soln should be equiv. to 1 ml As_2O_3 soln.

(e) *Arsenious oxide std soln.*—Dissolve 0.3241 g As_2O_3 in 25 ml 10% NaOH soln, make slightly acid with H_2SO_4 (1+6), and dil. with H_2O to 1 L.

24.007 DISTILLING APPARATUS

Use 800 ml Kjeldahl flask, *A*, distg tube, *B*, and 300 ml erlenmeyer, *C*, Fig. 44.

To prep. distg tube, bend 10–15 mm glass tube to acute angle of ca 70°. Draw longer arm, which is 15–20″ long, down to orifice of ca 3 mm. Fit shorter arm (ca 4″) with No. 7 rubber stopper previously boiled in 10% NaOH soln 15 min., and then in HCl 15 min. to remove most of S compounds that might be distd and react with the $KBrO_3$ soln. All-glass app. (Ramberg-Sjöström app., Fig. 65, **32.182,** for example) is very useful and will reduce blanks to min.

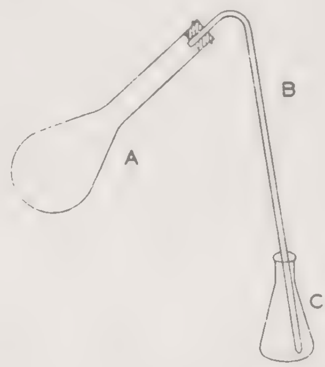

FIG. 44.—DISTILLING APPARATUS FOR DETERMINATION OF ARSENIC BY BROMATE METHOD

24.008 PREPARATION OF SAMPLE

Introduce suitable sample contg 0.005 grain (0.324 mg) or more of As_2O_3 into 800 ml Kjeldahl flask. Digest as in **24.003,** with following exception: Add exactly 20 ml H_2SO_4, or (rarely), if material is difficult to digest, exactly 25 ml, at beginning of digestion. After digestion is complete, add 50 ml H_2O and 25 ml of the NH_4 oxalate-urea soln, and boil until white SO_3 fumes extend up into neck of flask to decompose oxalates and urea completely. (Volatile intermediate products may titr. with $KBrO_3$. If available heat is insufficient to decompose these substances, it is preferable to evap. to fumes with H_2O alone. Hydrazine sulfate will destroy small quantities of oxides of N.)

24.009 ISOLATION

Add 25 ml H_2O to digested soln in Kjeldahl flask and cool to room temp. Add 100 ml H_2O to flask *C*. Add to soln in Kjeldahl flask 20 g NaCl

and 25 ml of the hydrazine sulfate-NaBr soln, and connect distg tube. Heat Kjeldahl flask over small well-protected flame, and distill into H_2O in erlenmeyer. (Heating is not intended to boil soln but to bring about evolution of HCl gas, which carries over $AsCl_3$. Absorption of evolved HCl gas by H_2O causes rise in temp., which indicates progress of distn.) Adjust flame so that temp. of distillate soln will rise to 90° in 9–11 min. and then discontinue distn. (Residual mixt. in flask should be not <55 ml.) If distn proceeds further, or larger quantity of H_2SO_4 than that specified is used in digestion, SO_2 is distd and is titrd as As.

24.010 DETERMINATION

Titr. distillate at once with std $KBrO_3$ soln, using 3 drops Me orange. (Single drops of indicator, **4.004**(**g**), but not exceeding 3, may be added during titrn as red color fades.) Toward end of titrn add $KBrO_3$ soln very slowly and with constant agitation to prevent local excess. End point is reached when single drop $KBrO_3$ just destroys final tinge of red. Compare color with H_2O in erlenmeyer. (Do not exceed end point, as indicator action is irreversible and back-titrns are not reliable. At proper end point, red color produced by 2 addnl drops Me orange should persist at least 1 min.) Correct results for vol. $KBrO_3$ used in blank detn (digest 5 g pure sucrose) with same reagents (same quantities) and regular distn procedure. (Blank titrn should be not >0.7 ml $KBrO_3$ soln. Method is accurate down to variations in blank, which should be not >0.1 ml when chemicals from same lot are used.) Should blank titrn be high or variable, test individual reagents for purity by $KBrO_3$ titrn and discard unsatisfactory ones. Test the H_2SO_4 by bringing 20 ml to boil, cooling, dilg with H_2O to 100 ml, adding little HCl, and titrg while hot. (It probably will furnish most of blank.) Select rubber stoppers carefully as they are often source of unsatisfactory blanks.

If high results, due to SO_2 produced during distn or to other reducing substances, are suspected, dil. titrd distillate to definite vol. and again det. As in aliquot by Gutzeit method, **24.003**-**24.005**. Positive test for sulfates in aliquot of titrd distillate indicates contamination with reduced S compounds and necessity for check on the As.

CADMIUM (*4*)—FIRST ACTION

24.011 PRINCIPLES

Method involves the dithizone technic. Sample is digested with H_2SO_4 and HNO_3. All reactive metals are then extd from the soln (after adjustment to pH ca 9) with dithizone-$CHCl_3$ reagent. Cu, Hg, and most of any Ni or Co present are removed by stripping the $CHCl_3$ soln with dil. HCl. Aq. layer, adjusted to 5% NaOH, is extd with dithizone-CCl_4 reagent. At this alky Zn, Pb, and Bi do not extract, whereas Cd dithizonate is relatively stable. Last 2 steps (stripping in dil. HCl and development of Cd dithizonate in 5% NaOH) are repeated. Cd is finally estimated photometrically as the dithizonate. Zn constitutes chief interference.

24.012 REAGENTS

(**a**) *Citrate.*—Diammonium salt or citric acid.

(**b**) *Chloroform.*—Distill from hot H_2O bath, collecting distillate in absolute alcohol in proportion of 10 ml alcohol to 1 L distillate. Shake receiver intermittently during distn.

(**c**) *Diphenylthiocarbazone* (*dithizone*), *twice purified.*—Purify as in **24.039**(**e**), but make only 3 dil. NH_4OH extns of the $CHCl_3$ soln. Carry thru, including H_2O-washing steps, and then repeat purification with 3 NH_4OH extns, pptn with dil. acid, etc. Instead of heating ext. to dryness, evap. spontaneously, and complete drying under vac. in bell jar overnight.

(**d**) *Carbon tetrachloride.*—Reflux vigorously on steam bath 1 hr with $\frac{1}{20}$ vol. 20% KOH in MeOH. Cool, add H_2O, drain off CCl_4 layer, and wash at least 3 times with copious vols of H_2O until alkali-free. Dry over $CaCl_2$, filter, and distill on hot H_2O bath. (Unless reagent is so purified, erratic Cd results may be obtained with some lots of CCl_4.)

(**e**) *Dithizone in carbon tetrachloride.*—20 mg/L CCl_4,(**d**). Prep. daily, as dil. solns of dithizone are unstable. (When many detns are to be made, dithizone reagent may be prepd by diln from 300 mg/L soln. Store concd reagent under 0.1*M* SO_2 soln in refrigerator.)

(**f**) *Dithizone in chloroform.*—1000 mg/L $CHCl_3$, (**b**), prepd as needed.

(**g**) *Sodium hydroxide soln.*—28%. Dissolve 28 g NaOH pellets in H_2O and dil. to 100 ml.

(**h**) *Absorbent cotton.*—Metal-free. If traces of metal are present, remove by digesting the cotton several hr with warm 0.2*N* HCl, filtering on büchner, and finally washing with copious vols of redistd H_2O until acid-free.

(**i**) *Cadmium std soln.*—Dissolve 1 g pure Cd in 20–25 ml HNO_3 (1+9), evap. to dryness, add 5 ml HCl (1+1), evap. to dryness, and then add several ml H_2O and again evap. to dryness. Dil. this $CdCl_2$ soln to 1 L. (1 ml = 1 mg Cd.) Prep. intermediate std soln of 100 mmg Cd/ml by dilg stock soln. Convenient final working std soln is 2 mmg Cd/ml. Add HCl, 15 ml/L, before dilg to vol. to give final acidity of ca 0.2*N*.

24.013 PREPARATION OF STANDARD REFERENCE CURVE

Prep. in duplicate 6 Cd stds contg 0, 5, 10, 15, 20, and 25 mmg Cd as follows: Add appropriate

vols std soln to Squibb-type separators (125 ml size is convenient), adjust to 40 ml with 0.2N HCl, add 10 ml of the NaOH soln (soln is then 5% with respect to NaOH) and 25 ml dithizone soln, **24.012**(**e**), shake vigorously exactly 1 min., let stand exactly 3 min., and filter org. layer thru pledget of the absorbent cotton, discarding first 5 ml. Fill absorption cell (10 mm length is convenient) and det. absorbance at 510 mμ. Plot standard curve or calc. reference equation by method of least squares, **24.045**(**b**).

24.014 PREPARATION OF SAMPLE

Use sample equiv. to 5–10 g of the product, calcd to dry basis. (Sample size is of concern only when comparatively large proportions of Mg and P are present.) Digest with 10 ml H_2SO_4 (1+1) and HNO_3 as needed. If sample tends to char rather than to oxidize evenly, add 5 or 10 ml more H_2SO_4. Continue digestion, adding HNO_3 as required, until digestion is complete and SO_3 is evolved. Cool, add 15 ml satd NH_4 oxalate soln, and again heat to fumes.

Fat in biological materials, such as liver and kidney, may cause bumping and frothing during digestion. If comparatively large samples of such materials are available, make partial digestion with warm HNO_3 until only fat remains undissolved. Cool, filter free of solid fat, wash residue with H_2O, make combined filtrate to suitable vol., and digest appropriate aliquots as above.

24.015 DETERMINATION

Dil. digest, **24.014**, with 25 ml H_2O, filter free from excessive insol. matter (sulfates or silica) if present, and transfer to separator marked at 125 ml, using addnl 10 ml portions H_2O for rinsing and completing transfer. Add 1–2 g citrate reagent, **24.012**(**a**), and 1 ml thymol blue indicator, **39.018**(**c**), and adjust to ca pH 8.8 by adding NH_4OH slowly, while cooling intermittently, until color of soln changes from yellowish green to greenish blue. Dil. to 125 ml mark with H_2O. Ext. vigorously with 5 ml portions dithizone soln, **24.012**(**f**), until $CHCl_3$ layer remains green. Then ext. with 3 ml $CHCl_3$.

Transfer all $CHCl_3$ exts to second separator previously wetted with 2–3 ml $CHCl_3$. Add to combined dithizone exts 40 ml 0.2N HCl, shake vigorously at least 1 min., and after layers sep., carefully drain $CHCl_3$ phase contg any Cu, Ni, Co, or Hg that may be present, and discard. Remove remaining droplets of dithizone by extg with 1–2 ml CCl_4, **24.012**(**d**), carefully conducting draining operation so that no acid enters bore or stem of separator, as its presence there would decompose in part Cd dithizonate subsequently formed and extd in next step.

Adjust aq. phase to 5% alky by adding 10 ml of the NaOH soln. Ext. Cd with 25 ml dithizone soln, **24.012**(**e**), shaking vigorously at least 1 min., and transfer to third separator previously wetted with 2–3 ml of the same soln. Repeat extn with addnl 10 ml portions of the dithizone soln until CCl_4 layer becomes colorless. Quantities of Cd usually found in foods or biological materials (ca 100 mmg) are completely removed by third extn.

To verify assumption that pale pink color persisting after third extn is due to Zn, transfer questionable ext. to fourth separator contg 5% NaOH soln, add several ml dithizone soln, **24.012**(**e**), and shake vigorously. If CCl_4 layer becomes colorless, original pink color was due to Zn and no further extns are necessary. If, however, pink color persists, indicating presence of Cd, add the ext. to contents of third separator, and continue extn.

Convert Cd and Zn dithizonates in third separator to chlorides by adding 40 ml 0.2N HCl and shaking vigorously at least 1 min. Carefully drain CCl_4 layer, which may contain traces of Co and Ni not removed in second step, and discard. Remove droplets of dithizone from aq. phase by rinsing with 1–2 ml of the CCl_4 and drain off as completely as possible but do not permit any acid to pass bore of separator. Again adjust alky to 5% by adding 10 ml NaOH soln, **24.012**(**g**). Wipe separator stems dry with cotton, **24.012**(**h**). Det. Cd present by adding exactly 25 ml dithizone soln, **24.012**(**e**), shaking vigorously exactly 1 min., permitting layers to sep. exactly 3 min., and continuing as in **24.013**, beginning "filter org. layer . . . " Calc. Cd in mmg by substituting absorbance in linear equation or from std curve.

NOTE: If photometric measurement indicates >25 mmg Cd, make first approximation by dilg dithizonate soln with CCl_4 and evaluating absorbance. For best results repeat analysis with wts or aliquots of samples contg not >25 mmg Cd; 30 mmg is upper limit of solubility of Cd dithizonate in 25 ml CCl_4. Therefore quantities >30 mmg are incompletely extd.

COPPER

Volumetric Method (5)—First Action

(Min. 1 mg Cu)

24.016 REAGENTS

(**a**) *Copper std soln.*—Dissolve 318 mg pure Cu in HNO_3 and evap. to dryness on steam bath. Add enough H_2O and few drops HOAc to dissolve the $Cu(NO_3)_2$ and again evap. to dryness on steam bath. Redissolve the $Cu(NO_3)_2$ as above and dil. to 1L.

(**b**) *Sodium thiosulfate soln.*—Dissolve 24.82 g $Na_2S_2O_3.5H_2O$ in 1 L CO_2-free H_2O to make ca 0.1N soln. Let stand, preferably ca 2 weeks. Prep. 0.005 or 0.01N solns by diln with CO_2-free H_2O. Stdze daily against std Cu soln as follows:

Place 20 ml std Cu soln in 100 ml erlenmeyer, add excess of NH_4OH, and continue as in **24.018**, beginning "and boil gently to drive off excess NH_3." 1 ml $0.01N$ $Na_2S_2O_3 = 0.6357$ mg Cu.

24.017 PREPARATION OF SAMPLE

Digest 50–100 g sample as in **24.003**, or ash it as in **24.040**.

24.018 DETERMINATION

Dissolve ashed sample in HCl and neutralize, or neutralize soln obtained by wet digestion with NH_4OH. Add 5 ml H_2SO_4, dil. to 200 ml, and boil 1 min. Add cautiously 10 ml hot satd $Na_2S_2O_3$ soln and continue boiling 5 min. (With larger quantities of Cu, ppt coagulates, and liquid becomes practically clear. Few ml 1% $(NH_4)_2SO_4$ soln may be added to hasten coagulation.) Filter ppt and wash 6 times with hot H_2O. Reserve filtrate for detn of Zn, if necessary.

Fold ppt within filter paper, place in small crucible, and ignite in elec. muffle at 500°. Treat residue with 1 ml HNO_3 (2+5) and dry on steam bath. Add 5 ml H_2O and again dry on steam bath. Add 20 ml H_2O and excess of NH_4OH, and heat on steam bath until Cu salts dissolve. Transfer to 100 ml erlenmeyer and boil gently to drive off excess NH_3. Make acid to litmus paper with HOAc (1+1), add 10 ml excess, boil soln 1 min., and cool to room temp. Add 2 g KI dissolved in enough H_2O to make final soln 50 ml, and titr. free I immediately with 0.01 or $0.005N$ $Na_2S_2O_3$ (according to quantity of Cu present, as shown by degree of blue color in ammoniacal soln) until end point is nearly reached. Add 2 ml starch indicator, **24.039(h)**, and continue titrn dropwise to disappearance of blue color. Compare with titrd std.

International Union of Pure and Applied Chemistry Carbamate Method (6)—First Action

24.019 PRINCIPLES

Method consists of wet digestion of sample with HNO_3 and H_2SO_4. Cu is isolated and detd colorimetrically at pH 8.5 as diethyldithiocarbamate in presence of chelating agent, ethylenediaminetetraacetic acid disodium salt (EDTA). Bi and Te also give colored carbamates at pH 8.5 but are decomposed to colorless compounds with $1N$ NaOH. Cu complex is stable. Range of color development is 0–50 mmg. Blank is ca 1 mmg Cu.

24.020 PRECAUTIONS

Clean glassware with hot HNO_3. Use white petrolatum to lubricate stopcocks of separators, and do not use brass chains. Purify H_2O and HNO_3 by distn in Pyrex.

24.021 REAGENTS

(**a**) *Sodium diethyldithiocarbamate (carbamate soln).*—Dissolve 1 g of the salt in H_2O, dil. to 100 ml, and filter. Store in refrigerator and prep. weekly.

(**b**) *Citrate-EDTA soln.*—Dissolve 20 g dibasic NH_4 citrate and 5 g ethylenediaminetetraacetic acid disodium salt (Eastman) in H_2O and dil. to 100 ml. Remove traces of Cu by adding 0.1 ml carbamate soln and extg with 10 ml CCl_4. Repeat extn until CCl_4 ext. is colorless.

(**c**) *Copper std soln.*—1 mg/ml. Place 0.2000 g Cu wire or foil into 125 ml erlenmeyer. Add 15 ml HNO_3 (1+4), cover flask with watch glass, and let Cu dissolve, warming to complete soln. Boil to expel fumes, cool, and dil. to 200 ml. Dil. 20 ml to 200 ml for intermediate std (0.1 mg/ml). Prep. working std, 2 mmg/ml, daily by dilg 5 ml intermediate std to 250 ml with $2.0N$ H_2SO_4.

(**d**) *Ammonium hydroxide.*—$6N$. Purify as in (**b**).

24.022 PREPARATION OF SAMPLE

Weigh sample contg not >20 g solids, depending upon expected Cu content. If sample contains <75% H_2O, add H_2O to obtain this diln. Add initial vol. HNO_3 to equal ca 2 times dry sample wt and 5 ml H_2SO_4, or as many ml H_2SO_4 as g dry sample, but at least 5 ml. Digest as in **24.003**.

When sample contains large amount of fat, make partial digestion with HNO_3 until only fat is undissolved. Cool, filter free of solid fat, wash residue with H_2O, add H_2SO_4 to filtrate, and complete digestion as above. After digestion, cool, add 25 ml H_2O, and remove nitrosylsulfuric acid by heating to fumes. Repeat addn of 25 ml H_2O and fuming. If after cooling and dilg, insol. matter is present, filter thru acid-washed paper, rinse paper with H_2O, and dil. to 100 ml.

Det. reagent blank.

24.023 ISOLATION AND DETERMINATION OF COPPER

Pipet 25 ml sample soln into 100 or 250 ml short-stem separator and add 10 ml citrate-EDTA reagent. Add 2 drops thymol blue indicator, **35.095(k)**, and $6N$ NH_4OH dropwise until color turns green or blue green. Cool, and add 1 ml carbamate soln and 15 ml CCl_4. Shake vigorously 2 min. Let layers sep. and drain CCl_4 through cotton pledget into g-s. tube or flask. Det. absorbance or transmittance in suitable instrument at ca 400 mμ.

If >50 mmg Cu is present in 25 ml aliquot, use smaller aliquot and dil. to 25 ml with $2.0N$ H_2SO_4. Highest accuracy is obtained at ca 25 mmg Cu level (absorbance ca 0.3 in 1 cm cell).

To test for Bi and Te return CCl_4 soln to separator, add 10 ml 5% KCN soln, and shake 1 min. If CCl_4 layer becomes colorless Bi and Te are absent.

If test is positive, develop color in another 25 ml aliquot as above (without KCN). Drain CCl_4 layer into second separator, add 10 ml 1*N* NaOH, and shake 1 min. Let layers sep. and drain CCl_4 into third separator. Again wash CCl_4 ext. with 10 ml 1*N* NaOH. Det. absorbance or transmittance of CCl_4 layer and convert to mmg Cu.

24.024 PREPARATION OF STANDARDS AND CALIBRATION CURVES

Transfer to separators 0, 1, 2.5, 5, 10, 15, 20, and 25 ml of std Cu soln (2 mmg/ml) and add 2.0*N* H_2SO_4 to make total vol. of 25 ml.

Add 10 ml citrate-EDTA reagent and proceed as in **24.023,** beginning "Add 2 drops thymol blue indicator . . ."

Plot absorbance against mmg Cu on ordinary graph paper. If readings are in % transmittance, use semilog paper, and plot transmittance on log scale. Since there is usually some deviation from linearity, read sample values from smoothed curve.

FLUORINE—OFFICIAL

24.025 PRINCIPLES

General method specifies ashing of sample with $Ca(OH)_2$ as F fixative, isolation of F by Willard-Winter distn (*7*) from $HClO_4$, and estimation in distillate by $Th(NO_3)_4$ back-titrn procedure (*8*). Technic and reagent concns are designed to handle conveniently not >10.0 mg F. Modifications of this general procedure, applicable to specific products, are described.

24.026 PRECAUTIONS AND INTERFERENCES

Control magnitude of detn blank by careful choice and purification of reagents (*see* **24.028**). With care, blank will be low (1–3 mmg F), but with low-F foods it may represent considerable part of total F detd. Hence it must be stable. Large part of it will be "distn blank" apparently resulting from F leached from glassware of still during distn. This blank can be minimized by preliminary treatment of still, **24.030,** and it should be possible to deduce and correct for an av. distn blank if stills of same material and design are routinely used; otherwise, each still must bear its special blank. New, unused stills will usually be found to exhibit high blank, which will diminish to constant low figure after several runs. They should not be used until analyst has assured himself, by means of several consecutive blank runs, that still is yielding not more than constant, low quantity of F.

Check ashing utensils by blank detns with fixative soln to ascertain whether or not they contribute appreciable F. Even Pt vessels may become contaminated (owing presumably to slight Ca content) if they have been used recently for HF volatilization of SiO_2. In addn, such blank detns are useful for testing reagents and app. used in method and also evaporators, hoods, muffles, and laboratory atmosphere for presence of F fumes and dust. If HF bottles are permitted in same laboratory, seal immediately after use; avoid contamination from roach powders.

Ordinary tap H_2O may be source of F contamination, since 1 ml H_2O contg 2 ppm F will contribute 2 mmg F if allowed to remain or to dry in still. Therefore routinely rinse all glassware (stills, flasks, burets, etc.) with H_2O, preferably redistd from alk. $KMnO_4$. Filter papers may contribute small (mmg) quantities of F, and glass filters are preferred if filtration is required in micro detns.

Interferences are gelatinous SiO_2, Al, and B compounds, which repress evolution of F as H_2SiF_6 in distn; materials such as nitrates, nitrites, peroxides, Cl, SO_2, and H_2S, which act upon indicator in titrn or otherwise interfere; halides (Cl), which distill to give excessive acidity in distillate; and phosphates and sulfates, which react with Th in titrn to give high results. Method is so designed that most of these interferences are automatically eliminated, but analyst should be on guard against their possible occurrence under unusual circumstances.

General Method

24.027 APPARATUS

(a) *Fluorine still.*—Claisen 100–125 ml distg flask is most practical for general work. It must be of Pyrex glass with auxiliary neck sealed off immediately above side-arm to prevent pocketing and refluxing of distillate. Still should be as small and simply designed as practicable; in fact ordinary distg flasks can be used for some work and they are slightly more efficient than Claisen type, except that there is more danger of spraying over of distg acid.

Equip still with dropping funnel and 0–150° thermometer, latter extending to within $\frac{1}{4}''$ of bottom of flask, so that bulb is immersed in boiling acid mixt. Acid-alkali washed beads, preferably Pyrex, should be on hand. Clean rubber stoppers by boiling in 10% NaOH soln. All-glass app. with ₸ accessories is convenient, especially in routine work, and eliminates need for rubber stoppers.

While not entirely necessary for heating still, use of Wood metal (50Bi, 25Pb, 12.5Sn, 12.5Cd)

bath, adequately shielded, will prevent undue decomposition of $HClO_4$ and aid materially in securing low blank and low-acid distillate; hence its use is strongly urged. If metal bath is used, do not immerse flask so deeply that bath level is above that of liquid in flask; if bath is not used, transite or asbestos shielding boards are essential, and flask should be heated thru small hole in such shield by low "clean" flame. (Purpose of bath and shielding boards is to prevent over-heating of upper still walls.)

At analyst's option, distg H_2O may be added as steam instead of thru dropping funnel; elec. boiler (*9*), Fig. 31, page 236, is convenient steam generator. If steam is used, inlet tube should dip below surface of liquid in still. One advantage in adding distg H_2O thru funnel is that last portions of rinse H_2O used in transferring an ash can be used in distn. If funnel plug is thinly notched with sharp file on either side of bore, dropping rate can be more easily controlled, and end of funnel stem need not extend into liquid in still. Still is used in conjunction with clean straight-tube condenser no longer than necessary for adequate cooling. (Vertical arrangement of condenser will conserve bench space.)

(**b**) *Nessler tubes.*—Tall form, 100 and 50 ml, g-s. type preferred. Matched in sets of at least 6. (100 ml size will be used more frequently in general method.)

(**c**) *Additional apparatus.*—In addn there will be required (*see* **24.026**) carefully cleaned and tested Pt, or well-glazed porcelain, dishes of at least 100 ml size; 150 ml vol. flasks, or if these are not available, 200 ml size; and 10 ml burets (conveniently automatic) to deliver various solns required in distn and titrn. Overhead radiant heater will be found invaluable for drying and preliminary charring of samples, especially those of high-sugar type.

24.028 REAGENTS

(**a**) *Lime suspension.*—Carefully slake ca 56 g (1 mole) low-F CaO (ca 2 ppm F) with ca 250 ml H_2O, and add 250 ml 60% $HClO_4$ *slowly* and with stirring. Add few glass beads and boil down to copious fumes of acid; then cool, add 200 ml H_2O, and boil down again. Repeat diln and boiling down once more; cool, dil. considerably, and filter thru fritted glass filter, if pptd SiO_2 appears. Pour clear soln, with stirring, into 1 L NaOH soln (10 g/100 ml), let ppt settle, and siphon off supernatant. Remove Na salts from ppt by washing 5 times in large centrifuge bottles, shaking up mass thoroly each time. Finally, shake ppt into suspension and dil. to 2 L. Store in paraffined bottles. (100 ml of this suspension should give no appreciable F blank when evapd, distd, and carried thru titrn procedure described below.) Always shake suspension well before use.

(**b**) *Perchloric acid soln.*—60%. Dil. $HClO_4$ with 3–4 vols H_2O and boil down to original vol. Do not fume strongly. Repeat, and store in Pyrex. (Prepd acid should be Cl-free by test.)

(**c**) *Sulfuric acid soln.*—Carefully mix equal vols H_2SO_4 and H_2O, boil down to fumes, cool, dil. *carefully*, boil down once more, and dil. to 1+1 vol.

(**d**) *Silver perchlorate soln.*—50 g/100 ml.

(**e**) *p-Nitrophenol indicator.*—0.5% alc. soln.

(**f**) *Potassium hydroxide soln.*—Exactly 0.05*N*.

(**g**) *Potassium chloride soln.*—0.05*N*. 3.727 g/L.

(**h**) *Hydroxylamine hydrochloride soln.*—1.0%.

(**i**) *Hydrochloric acid soln.*—Exactly 0.05*N*.

(**j**) *Alizarin indicator.*—0.01% aq. soln of sodium alizarin sulfonate (Alizarin Red S).

(**k**) *Potassium fluosilicate.*—If pure K_2SiF_6 is not obtainable, prep. as follows: Add, thru dropping funnel, satd soln of NaF, or suspension of crude K_2SiF_6, into 500 ml Claisen distg app. contg 60 ml H_2SO_4 (1+1), some glass beads, and 10–20 g powd. SiO_2 (or glass) kept at boiling temp. of 120–125°. Conduct distillate into soln of ca 25 g pure KCl in H_2O, held at simmering temp. on hot plate so that vol. of distillate does not become excessive. If necessary, add more H_2O to mixt. from dropping funnel in side-neck of still. Regulate rate of addn of fluoride to still and temp. of condensing H_2O so that side-arm and condenser do not become clogged with evolved H_2SiF_6, which tends to lodge as gelatinous mass. K_2SiF_6 is formed in receiver and altho entirely cryst. it assumes appearance of gelatinous mass.

When substantial quantity collects, pour contents of receiver into large centrifuge bottle and wash repeatedly by centrifuging (shaking up ppt thoroly each time), until washings are Cl-free by test. Collect on büchner and either air-dry or bring to constant wt *in vacuo* at 50–70°.

Det. purity by Travers titrn, **4.018**, at boiling temp. with 0.2*N* NaOH (1 ml = 0.01102 g K_2SiF_6); also by conversion to K_2SO_4 by treating 0.3–0.4 g in deep Pt dish with little H_2O, then H_2SO_4 plus little HF, fuming off excess acid *carefully* (if overheated, mixt. has tendency to spatter), and heating to constant wt of K_2SO_4 at 650°. With glass app. entirely pure product is not usually obtained, as some contamination with SiO_2 results from leaching effect of vapors on condenser. Pure product can be obtained by use of Pt still. Dilg 0.9662 g pure K_2SiF_6 to 1 L gives stock soln contg 0.5 mg F/ml. Much more will not dissolve. Prep. such soln, correcting this wt of 0.9662 by purity factor of the K_2SiF_6 (figure for purity obtained from av. of 2 above methods of

assay). Soln keeps indefinitely in paraffined bottle.

Prep. soln *used in titrn*, **24.031**, by dilg 20 ml of this stock soln to 1 L (1 ml = 10 mmg F). It will retain its strength several weeks in ordinary volumetric ware.

(l) *Thorium nitrate soln.*—0.25 g $Th(NO_3)_4 .12H_2O$ or 0.20 g $Th(NO_3)_4.4H_2O$/L. Check titer against std (10 mmg/ml) F soln as follows: Measure 10, 20, 30, etc., up to 80 mmg F into 100 ml Nessler tubes, and add 4.00 ml of the 0.05*N* HCl (2.00 ml if 50 ml Nessler tubes are used, and carrying range to only 50 mmg F for this size tube) *(10)*. Dil. mixt. to ca 80 (or 40) ml mark and add 1.00 ml 1.0% $NH_2OH.HCl$ soln. Mix; then add exactly 2.00 ml alizarin indicator (or 1.00 ml for smaller tube) and measure in the Th soln from buret, mixing frequently until, when sighting down tube toward white reflecting surface, incipient pink or salmon pink color is observed. Add little H_2O occasionally so that soln is nearly to mark as end point is approached. Finally, make exactly to mark and mix thoroly before checking final end point. Do not shake tubes violently (5–6 gentle inversions are enough).

Make effort to secure end point shade intermediate between yellowish-green of acid indicator and reddish-purple of fully developed Th lake. Complete series and plot ml Th soln against ml std fluoride to obtain rough equivalence curve for 2 solns. Depending upon quantity of F known to be present, add the Th soln in 1–2 ml portions at first, with final addns of 0.25 ml.

24.029 PREPARATION OF SAMPLE

Methods of sample prepn are designed to furnish representative sample in workable quantity of material and to obtain sample in condition for final distn. Mineralization by ashing is usually involved. Some mineral food products can be dissolved in and distd from $HClO_4$, **24.030**, provided no interferences appear in final distillate.

In general, 20 g or more of dry material, 50–100 ml liquid samples, and 50–100 g undried food products or plant material can be taken for analysis, depending upon expected F content and interferences, such as excessive Cl, which use of large samples may introduce. For reasonable precision in analysis of low F foods, sample should be sufficient to yield titer of at least 0.5 ml for aliquot taken in final titrn. However, it may not always be possible to handle this quantity of material. If adequate grinding and mixing equipment is available, it is often feasible to prep. large quantities of material (vegetables, mixed foods) and to take aliquant portions for analysis *(11)*.

Dry plant materials, feeds, bone meal, etc., can be ground to convenient size in Wiley mill and thoroly mixed before sample is taken. Following special methods for certain products are indicated:

(**a**) *Direct ashing.*—Applicable to fibrous (not highly fatty) food materials, liquid samples, and in general to all foods that can be thoroly wet with aq. fixative soln. This procedure will apply to majority of food products.)

Weigh suitable portion of prepd sample into clean Pt dish and add 25 ml $Ca(OH)_2$ suspension. (Porcelain casseroles or dishes are second choice because they may contribute small quantities of F and Al_2O_3 to sample). Mix in $Ca(OH)_2$ suspension with glass rod, adding addnl H_2O if necessary; rinse and remove rod. Dry *thoroly* on steam bath or in hot air oven; then slowly char sample by heating over low flame or elec. plate with thermostat. Overhead radiant heater is convenient for both drying and charring sample. Control excessive swelling of high sugar foods by playing small flame over surface of sample from time to time, and char these products *slowly* so that excessive acidity is not generated. When sample is charred past danger of catching fire, ash in muffle at 600°. (For very small samples and min. blanks it may be advisable to cover ashing vessel with inverted Pyrex petri dish while ashing.)

When clean ash is obtained, cool dish and wet ash with ca 10 ml H_2O. (Small quantity of unburned C does not interfere but if much is apparent, dry down and repeat ashing.) Cover dish with watch glass and cautiously introduce under cover just enough of the $HClO_4$ soln to dissolve the ash. Rinse down cover with little H_2O and transfer soln to freshly prepd F still, **24.030**, thru long-stem funnel. Rinse dish with remainder of distg acid, using ca 20 ml in all, and adding and transferring in several small portions. *Do not prolong transferring operation.* Finally rinse funnel and stirring rod into dish, assemble still, and complete rinsing of dish with several small portions of H_2O, pouring these into dropping funnel of still. If distg H_2O is added as steam, **24.027**(**a**), rinse dish with little addnl H_2O and add directly to acid mixt. in still, but avoid excessive initial vol. Add ca 6 Pyrex beads and enough $AgClO_4$ soln, **24.028**(**d**), to ppt all Cl. (Reasonable excess of $AgClO_4$ does no harm; enough solid Ag_2SO_4 may also be used.) Proceed as in **24.030**.

(**b**) *Preliminary distillation.*—(Necessary with certain products high in phosphate, such as Ca phosphate and bone meal, in order to eliminate distd H_3PO_4 that may be present in appreciable quantities in first distillates. Also advisable with certain excessively fatty materials that may not be thoroly wet with the $Ca(OH)_2$ fixative, thus causing F loss in direct ashing method.)

(1) *For inorganic phosphatic materials, such as Ca phosphate.*—Weigh sample, usually 10 g, into

still; add few glass beads, enough $AgClO_4$ to ppt possible Cl, and ca 20 ml of the $HClO_4$. (If the inorg. phosphatic material does not contain excessive Ca (enough to cause heavy ppt of $CaSO_4$ in still), use similar quantity of the $1+1$ H_2SO_4.) Distill at 135–140°, collecting ca 200 ml distillate. (For this preliminary distn, extreme care in securing low-acid distillate is not essential.) Evap. distillate to dryness in Pt dish after addn of excess $Ca(OH)_2$ suspension, assuring alk. conditions by testing with drop of phthln. (If H_2SO_4 is used in this preliminary distn, add to distillate few drops of *F-free 30% H_2O_2* to oxidize possible sulfites.) Heat dried residue at 600° few min. to destroy indicator residues and possible Cl-contg compounds. Transfer contents of dish to freshly prepd still, **24.030**, with the 20 ml distg $HClO_4$ as in (**a**), and proceed with final distn as in **24.030.**

Take 20 ml samples of sirupy H_3PO_4 and collect at least 300 ml first distillate at 135°, letting the H_3PO_4 function as its own distg acid. (More distillate is necessary because the H_3PO_4 is less effective as F distg acid.) Neutralize with $Ca(OH)_2$ suspension, evap. to dryness, transfer to prepd still as above, and proceed as in **24.030.**

(2) *For organic phosphatic materials, such as bone meal, feed supplements, etc.*—As preliminary ashing treatment to destroy most of org. matter, moisten sample with enough $Ca(OH)_2$ suspension, dry, char, and heat 2–3 hr at 600°. Transfer ashed material to still, which contains several beads and enough $AgClO_4$ to ppt Cl, with 20 ml of the distg acid ($HClO_4$ or H_2SO_4, depending on Ca content of sample) as in (**a**), and continue as in (**b**)(1), "Distill at 135–140° . . . "

Certain organic phosphatic materials (small samples of bone, 2–5 g, such as entire bones of small test animals) *in which quantity of organic matter is not excessive,* may be distd directly as in (**b**)(1) without preliminary ashing. If sample contains appreciable Ca (bone samples), use $HClO_4$ with reasonable precaution; if org. phosphatic material does not contain excessive Ca, use $1+1$ H_2SO_4. In either case add more $Ca(OH)_2$ to first distillates and ash for longer periods to completely destroy distd organic matter (fatty acids). Transfer contents of dish to freshly prepd still, **24.030,** with the 20 ml $HClO_4$ as in (**a**) and proceed with final distn, **24.030.**

Baking powders (Ca phosphate and combination types): Place 10 g sample in deep, covered Pt dish or casserole and slake cautiously with ca 20 ml $Ca(OH)_2$ suspension. After action subsides, rinse cover, dry contents of dish *thoroly,* and ash 2–3 hr at 600°. Cool dish and, because of excess of carbonate in ash, treat it with several small portions of warm H_2O, breaking up with flat-end stirring rod, and transfer leachings to still. Then transfer remaining contents of dish with the 20 ml $HClO_4$, avoiding excessive effervescence when acid is added to carbonate soln in still. Add several glass beads and enough $AgClO_4$ soln, and proceed as in (**b**)(1), "Distill at 135–140° . . . " With *combination* or *Na Al sulfate* baking powders, collect at least 400 ml preliminary distillate, (**b**)(4).

Use of special still trap makes possible analysis of highly phosphatic *inorg. or thoroly ashed* materials, and phosphoric acids, with single distn. Special trap, or scrubber, consists of 12–15 g small, hollow glass beads supported in side-neck of the 125 ml Claisen flask by several indentations punched in side wall, and capped by glass disk or inverted bottom of 15 mm test tube. After construction of the glass-bead scrubber, side-neck is sealed off immediately above outlet tube. (Beads in scrubber must be wet with little H_3PO_4 (by tipping flask) before distn to furnish liquid acid phase.) Take 20 ml sirupy H_3PO_4, by itself, and 10 g samples Ca phosphate with 20 ml of the $HClO_4$, for the distn, and collect at least 400 ml distillate at 135°. With single distn observe precautions outlined in **24.027**(**a**), and also in **24.030,** regarding neutralization of final distillates. (Distillates should show practically no acidity.) Presence of only *traces* of distd H_3PO_4 will vitiate titrn; as little as 20 mmg P_2O_5 will definitely interfere. Accordingly, if single distn procedure is to be applied with confidence, it is necessary to test distillates obtained from phosphatic materials, by means of the special still, for presence of this interference.

For convenient test utilizing Schricker reagent (*12*), add 5 ml of $1+9$ diln of this reagent to 45 ml distillate in 50 ml cylinder or Nessler tube, mix, and immerse in steam bath 5–10 min. Compare against blank by sighting down tube. Blue or blue-green color indicates phosphate, and as little as 5 mmg (as P_2O_5) is readily detected. If distillate shows traces, make sure that such quantities are below interference level of 15 mmg in titrn aliquot before titrg addnl portions of distillate. (Test with Schricker reagent is also useful in usual double distn where phosphate interference is possible. Use of the special trap will save time where highly phosphatic materials are handled routinely, but it is not justified in ordinary work because of poor efficiency owing to excessive refluxing in distn.)

(3) *For excessively fatty and oily food materials (oil-packed foods, certain meats, etc., also entire undried and unground organs of test animals).*—If there is danger of F loss thru incomplete wetting with $Ca(OH)_2$ fixative soln, handle as follows: Weigh appropriate quantity of sample, usually 10–25 g, into still, and add Ag (preferably 0.1–0.2 g solid Ag_2SO_4), several glass beads, and 20–25 ml of the $1+1$ H_2SO_4. Distill at 130–135° and collect 200–250 ml distillate in beaker or open vessel. If

foaming is excessive, increase quantity of distg acid, and where necessary use larger (250–300 ml) still. If larger still or more acid is used, collect proportionately more first distillate. (Oil or fat of many of these products will tend to prevent foaming; and in some instances use of ca pea-size piece of pure paraffin is addnl aid.)

Oxidize distillate in cold by cautious addn of 2–3 ml *F-free 30% H_2O_2* to remove sulfites, let stand few min., and evap. portionwise in Pt dish contg excess (10–15 ml) $Ca(OH)_2$ suspension. Ash residue at 600° until clean. Proceed as in (**b**)(1), beginning "Transfer contents of dish to freshly prepd still . . ."

Handle pure oils by similar procedure: Use 10 g sample with 25 ml 1+1 H_2SO_4 and carry temp. at first to ca 170° to saponify; then carefully bring temp. down to 140° with distg H_2O and collect 250 ml or more of distillate. (It will probably be necessary to use higher reading thermometer for this procedure.) Oxidize distillate with 30% H_2O_2 and evap. to dryness after adding excess $Ca(OH)_2$ suspension. Ash at 600° and after brief preliminary ash period remove dish, add little H_2O plus addnl 1–2 ml of the H_2O_2 to remove sulfides, dry, and complete ashing. Proceed as in (**b**)(1), beginning "Transfer contents of dish to freshly prepd still . . ."

(4) *For aluminum and boron compounds.*—Al and B repress evolution of F. Isolate F by preliminary distn at elevated temp. For this purpose, weigh sample, usually 5–10 g, into still, add 25 ml of the 1+1 H_2SO_4, and conduct first distn at 160–165° (special thermometer), collecting 300 ml distillate. Oxidize distillate with 30% H_2O_2 as above, evap. in Pt dish with excess $Ca(OH)_2$ suspension, ash briefly at 600°, and proceed as in (**b**)(1), beginning, "Transfer contents of dish to freshly prepd still . . ."

24.030 FINAL DISTILLATION

Always make final distn from $HClO_4$, and take precautions to secure low acid distillate, **24.027**(**a**). Since interferences, such as org. matter, phosphate, sulfate, etc., must be absent from distillate, make distn with careful temp. control in presence of enough Ag salt to repress HCl evolution (**24.026**). It is well to check distillates for presence of possible phosphate as in **24.029**(**b**)(2), and where advisable, as in (**b**)(4), to test for sulfate with little dil. $BaCl_2$ soln. $HClO_4$ used in final distn is usually used in transferring ash to still, **24.029**(**a**). Few acid-alkali washed beads are used to control bumping. (Use of powd. SiO_2 does not appear necessary for microdetn.)

To promote better recoveries, and to minimize and render constant distn blank discussed in **24.026** and **24.031**, prep. still by special cleaning process before this transfer by treating it with *hot* 10% NaOH soln after each run, flushing out with tap H_2O, and then rinsing with distd H_2O. Occasionally (at least once a day, and especially after it has stood idle for any length of time), give still addnl treatment by boiling down 15–20 ml of the 1+1 H_2SO_4 until still is filled with fumes. Cool, pour off acid, treat with the 10% NaOH soln, and *thoroly* rinse out. (Cleaning should be especially meticulous after high-F or high-SiO_2 samples have been distd, and in such cases condenser should also be cleaned.)

At this stage prepd sample has been transferred to specially treated still, as directed above, for final isolation of F. Begin distn, and when temp. reaches 137°, keep at this point (±2°) by adding H_2O from dropping funnel, **24.027**(**a**). Heat still at such rate that all distns require ca same time. (This promotes uniformity in blank correction.) Catch distillate in 150 or 200 ml vol. flask. After few ml distillate collects, add 1–2 drops *p*-nitrophenol indicator, **24.028**(**e**), and keep distillate alk. to this indicator (faintest perceptible yellow) by adding drop or two of the 0.05*N* KOH from 10 ml buret from time to time during distn while swirling receiver. So regulate this addn of alkali that distillate is neutralized (within 1 drop of alkali) as it approaches the mark. Note carefully vol. alkali used. Dil. distillate to mark and mix thoroly. Do not let F distillate stand more than few min. before neutralizing.

If sample contains such large quantities of Cl that bumping in still cannot be controlled, dissolve ash of another sample, and acidify *slightly* with $HClO_4$. Dil. considerably and ppt Cl in dish with the $AgClO_4$ soln, avoiding large excess. Filter thru glass filter, wash ppt *thoroly* with hot H_2O, and evap. filtrate and washings to dryness after adding excess (to alky) of the $Ca(OH)_2$ suspension. Transfer residue to still with the $HClO_4$ and repeat distn as above.

24.031 TITRATION

Place aliquot of final distillate in Nessler tube and mark "*S*" (sample). (Optimum F content for titrn is 60–70 mmg for 100 ml Nessler tubes and 30–40 mmg for 50 ml size, and it is well to make exploratory titrn on small aliquot to check approximate F content of distillate. Larger tubes are necessary for precise results on low-F foods.)

Add the 0.05*N* HCl, 4.00 ml for 100 ml tubes and 2.00 ml for 50 ml size, and 1.00 ml of the $NH_2OH.HCl$ soln. (For routine work *with 100 ml tubes* the acid and $NH_2OH.HCl$ can be blended as 0.04*N* HCl made to 0.2% concn with NH_2OH .HCl, and proper quantity of both reagents added to tubes in single operation with 5 ml pipet.) Dil. to ca 90 (or 40) ml, mix well, then add proper quantity of the alizarin indicator (2.00 or 1.00 ml),

and mix again. Always add and mix in the $NH_2OH.HCl$ before adding indicator.

Prep. blank tube "B" by adding proper quantity of HCl and $NH_2OH.HCl$, and quantity of the 0.05*N* KCl soln representing same proportion of total vol. of 0.05*N* KOH used to neutralize distillate as aliquot vol. taken for the sample tube represents of total distillate vol. (Thus, if 1.50 ml 0.05*N* KOH was used to neutralize distillate of 150 ml and aliquot taken for tube "S" was 75 ml, add 0.75 ml of the 0.05*N* KCl to tube "B.") Dil. and mix, allowing slightly more headspace than in sample tube. Then add proper vol. alizarin indicator and mix.

Measure Th soln into tube "S," mixing between addns, until end point of about proper shade is reached. Dil. to mark, mix, and check this end-point shade. Note from curve, **24.028(l)**, approx. vol. std F soln corresponding to this vol. Th soln, and add ca 0.5 ml *less* than this quantity of std F soln to "B." Mix in; then add exactly same vol. Th soln as was added to "S," duplicating approx. increments in which it was added and number of mixings. Dil. nearly to mark and compare colors of "S" and "B." (If vol. std F soln added to "B" was properly chosen, this tube should be only slightly pinker in shade than sample tube.)

Bleach "B" tube to exact match with tube "S" by adding more std F soln to former in increments of 1–2 drops, mixing gently between addns. Dil. to mark for final comparison and observe usual precautions of letting bubbles subside and of transposing tubes when final comparisons are made. (At match-point, F content of tube "S" equals quantity added to tube "B.") Check this end point by adding 1–2 drops excess of std F soln to tube "B." Distinct over-bleach should develop.

Repeat titrn on aliquots of different size to obtain total quantity of F distd. If time is available, repeat entire detn with different wt sample.

For precise work, evaluation of reagent and of distn blank is necessary, **24.026.** Det. distn blank by making several distns with prescribed quantities of $HClO_4$ and $AgClO_4$ solns from freshly cleaned still, titrg distillate as above with as large aliquot as practicable. Av. of values found should be not >2–3 mmg F. If quantities found by individual blank detns are too small to be detd accurately, make 5 or more sep. distns and evap. distillates, 150 ml each time, successively in same Pt dish for final distn and average blank figure. Distn and total detn blanks can usually be *combined* by carrying run (with same quantities of reagents and similar evapn and ashing treatment) thru entire procedure. Reagents and manipulations should increase distn blank but little.

Calc. total quantity of F distd from quantity found in aliquot titrd, subtract proper blank, and refer net figure to wt sample taken. If double distn procedure was used, make appropriate blank correction.

Rapid Method Restricted to Fluoride Residues on Apples and Pears

24.032 PRINCIPLES

Acid filtrate from strip soln of apples and pears prepd with HCl rinse and acidification, **24.052,** is used. Aliquot of filtrate is oxidized colorless with $KMnO_4$, soln is then reduced with NH_2OH, and subaliquot is back-titrd in Nessler tubes; $Zr(NO_3)_4$ is used in titrn, with purpurin (1,2,4-trihydroxyanthraquinone) as indicator (*13*). Principle of back-titrn, as applied here, is similar to that used in general method where $Th(NO_3)_4$ and alizarin occupy similar roles. Provision is made for removal of interfering anions, and high acidity used in titrn minimizes interference of metals that would otherwise lake with indicator.

24.033 APPARATUS

Nessler tubes.—50 ml g-s., tall-form, matched for height and color (*see* **24.036**).

24.034 REAGENTS

(**a**) *Mixed nitrate soln.*—Dissolve 3.0 g $Ba(NO_3)_2$ and 2.0 g $Th(NO_3)_4.4H_2O$ in H_2O, and dil. to 100 ml.

(**b**) *Potassium permanganate soln.*—Satd; ca 6%.

(**c**) *Hydroxylamine hydrochloride soln.*—5%.

(**d**) *Ferrous chloride soln.*—Dissolve ca 1.0 g Fe powder or wire in 50 ml HCl (1+1), dil., and filter into 500 ml vol. flask. Add few ml of the $NH_2OH.HCl$ soln and dil. to mark. Dil. still further before use, if desired.

(**e**) *Purpurin indicator.*—0.01% w/v in alcohol. Dissolve 25 mg pure 1,2,4-trihydroxyanthraquinone in alcohol, heating if necessary, and dil. to 250 ml with same solvent. *Prep. fresh weekly.*

(**f**) *Zirconium nitrate soln.*—Dissolve 1.50 g $Zr(NO_3)_4.5H_2O$ in H_2O, acidify with 20 ml HCl, and dil. to 1 L. Filter if not clear.

(**g**) *Fluoride std soln.*—Dil. stock soln of pure NaF so that 1 ml = 54.5 mmg F.

24.035 DETERMINATION

Place 20 ml well-mixed acid strip filtrate, **24.052,** in 50 ml vol. flask. Add 2.0 ml of the mixed nitrate soln, then 4.0 ml of the $KMnO_4$ soln. Rinse down neck of flask with little H_2O and place on active steam bath 5 min. Remove flask, and while still hot, add the $NH_2OH.HCl$ soln from buret, slowly and with swirling, until MnO_2 is dissolved and soln is colorless. Add ca 0.5 ml of this reagent in excess. (Appreciable phosphate is revealed as flocculent $Th_3(PO_4)_4$, and sulfate as ppt with Ba. Sometimes $KMnO_4$ is occluded in sulfate

and/or phosphate ppt, and pink color tends to persist but does not interfere.) Cool, dil. to mark, and filter. (Filtrate must be clear. If there is perceptible turbidity, return filtrate thru filter several times if necessary, until filtrate is *brilliant.*) Pipet 25 ml clear filtrate into Nessler tube and mark "*S*."

For blank or comparison tube use 25 ml "blank" soln, contg reagents used in method, prepd as follows:

Dil. 50 ml 10% Na oleate soln, **24.039**(o), 50 ml 30 g/100 ml NaOH soln, and 15 ml HCl to 1 L. Acidify portions with $\frac{1}{10}$ vol. HCl as if soln were an actual "strip," and filter, refiltering until filtrate is perfectly clear. (Chilling soln and shaking vigorously will "churn" pptd oleic acid and aid in obtaining clear filtrate.) Carry 20 ml portions of acidified filtrate thru method exactly as above. (It is best, in order to duplicate more closely conditions of actual detn, to use 50 ml vol. flasks and 20 ml aliquots in preference to working up larger aliquots with correspondingly larger quantities of reagents. After being dild to vol. and filtered, blank solns may be combined to form supply of "blank"; 10 portions worked up as above yield ca 500 ml "blank," or enough for ca 20 detns.)

Add 25 ml of this "blank" to second Nessler tube, "*B*," and to both tubes "*S*" and "*B*" add 15.0 ml HCl measured as carefully as possible from graduate. (Always add acid to soln instead of vice versa.) Mix, and match tubes for color. "*S*" tube will usually be found to have slight greenish tint in comparison with "*B*" tube, due presumably to traces of Fe. Balance both tubes to same shade by adding the $FeCl_2$ soln dropwise to appropriate tube and mixing. *This operation must be done carefully.* When tints are indistinguishable, add exactly 1.00 ml of the purpurin indicator to each tube. Mix; then add 1.50 ml of the Zr soln to each tube from 10 ml buret, and mix. Do not shake tubes violently when mixing in reagents; 4 or 5 gentle inversions are enough. Observe color difference, if any, between 2 tubes when looking down their length toward white reflecting surface. If there is no appreciable difference *after 5 min.*, F content of sample is negligible. If color of tube "*S*" is yellower, presence of F is indicated. In this case, add addnl quantities of the $Zr(NO_3)_4$ soln to tube "*S*" until its color matches about that of tube "*B*" (to nearest 0.5 ml of the Zr soln). Dil. "*S*" to mark and mix.

Now add to "*B*" exactly same total vol. Zr soln as was added to tube "*S*," mix, and let tube stand 2 min. for lake to develop fully. Back-titr. std F soln into "*B*" from 10 ml buret until tubes match, frequently mixing, and dilg nearly to vol. as end point approaches. Add NaF soln in increments of ca 0.1 ml at this stage, and observe usual precautions of transposing tubes and letting bubbles subside when making comparisons. Dil. to mark for final comparison. Check end point by adding 0.1–0.2 ml of the std F soln in excess. Distinct overbleach should develop.

For sample wt of 1 kg and aliquots prescribed above, each ml std F soln consumed in back-titrn is equiv. to F content on fruit sample, *removable by the solvent treatment,* of 3.0 ppm. Correct result obtained in titrn by sample wt ratio. (Thus, titer of 3.27 ml std F soln, with 1.40 kg sample (ca 10 fruit), represents F content of 7.0 ppm. Vol. restrictions of 50 ml Nessler tube will allow estimation of spray residue content up to ca 11 ppm F.) If calibration mark is exceeded in back-titrn, use 10 ml aliquot of acid filtrate in tube "*S*," and dil. to 25 ml with "blank" soln, correcting titer of std F soln by appropriate factor.

24.036 NOTES ON RAPID METHOD

G-s. Nessler tubes are almost essential with concd acid prescribed in this detn and are likewise desirable in general method for F with Th and alizarin, **24.031.** Analysts familiar with Th-alizarin back-titrn method should have no difficulty with Zr-purpurin titrn. With latter, however, color changes are not so apparent and titrn is less sensitive. However, with careful work, results accurate to at least 0.5 ppm may be expected.

Indicator color at prescribed acidity is yellow, and fully laked indicator is orange-red. This contrasts with Th titrn where corresponding range is from yellowish-green to reddish-purple. Hence in rapid method choice of end point involves discrimination between varying shades of orange. Addn of the 1.50 ml Zr soln to tube "*B*" at start is merely to provide intermediate shade of orange to guide analyst in quantity of Zr to be added to tube "*S*." Analysts may prefer to work with redder or yellower end point shade. In any event, make number of titrns by adding varying quantities of std F soln as unknowns to Nessler tubes and carrying thru back-titrn as above, for purpose of learning color changes involved. Pure aq. solns instead of "blank" may be used, with acidities of 20 ml HCl/50 ml.

Accuracy of results with rapid method presupposes complete removal of spray residue F by solvent process and good accuracy (not necessarily precision) in titrn. These conditions may not always hold; unless carefully done, solvent method may not be entirely effective, and results on strip solns contg known quantities of F have tended to be slightly low. Hence accuracy above 95% is not to be expected with this method.

LEAD (*14*)—OFFICIAL

24.037 PRINCIPLES

General method calls for ashing, **24.040,** sepn of Pb, either as dithizone complex, **24.042,** or as

sulfide, **24.043**, followed (depending upon quantity) by electrolytic detn, **24.044**, or by colorimetric dithizone detn, **24.045**, in comparator tubes, or with spectrophotometer. Subject of interference is treated separately, **24.046–24.048**, and analyst should familiarize himself with details of these sections before applying method. Special methods of sample prepn are presented in **24.049–24.051**.

24.038 PRECAUTIONS

Analyst should decide whether nature of detn requires unusual care in purification of reagents, or whether blank detn will suffice. Smaller the quantity of Pb to be detd, greater the care required in reduction of blank (*see also* **24.045**).

To test suitability of reagents place 10–15 g solid reagents dissolved in redistd H_2O or 15–20 ml concd acids previously neutralized with redistd NH_4OH in separator and add enough Pb-free citric acid to prevent pptn by NH_4OH of Fe, Al, alk. earth phosphates, or other substances. Make soln ammoniacal and add 2–3 ml 10% KCN soln. Shake soln with ca 5 ml dithizone soln, **24.039**(e) (5–10 mg/L). If lower layer is green, transfer it to another separator and ext. excess dithizone with NH_4OH (1+99) to which has been added drop of KCN soln. If $CHCl_3$ layer is colorless, consider test negative for general analytical purposes.

When special purification becomes necessary, redistill H_2O (distd H_2O stored in Sn-lined tanks usually contains Pb and Sn), HNO_3, HCl, HBr, Br, and $CHCl_3$ in all-glass stills (preferably Pyrex). Prep. NH_4OH by distg ordinary reagent into ice-cold redistd H_2O. If stills are new, steam them out with hot HCl or HNO_3 vapors to remove "surface" Pb. (Subsequent distillates may not be totally Pb-free.)

Purify citric acid, NaOAc or NH_4OAc, $Al(NO_3)_3$, $Ca(NO_3)_2$, and Na_2SO_4 by pptg the Pb from their aq. solns with H_2S, using 5–10 mg $CuSO_4$ as coprecipitant (citric acid and $Al(NO_3)_3$ solns require adjustment with NH_4OH to pH 3.0–3.5, bromophenol blue indicator). Filter (fritted glass filter is most convenient), boil filtrates 20 min. to expel excess H_2S, and refilter if necessary to obtain brilliantly clear solns. Purify other reagents by recrystn.

Store redistd acids or purified solns of reagents in resistant glass containers of min. Pb content (Pyrex is suitable), carefully cleaned of surface Pb with hot HNO_3. Paraffin-lined bottles may be used for alk. reagents.

Clean new glass and chemical ware carefully with hot 10% NaOH soln followed by hot HNO_3, and use only for Pb detns.

In prepn of samples for analysis, avoid Pb contamination. If mixing or grinding is necessary, use porcelain mortar if possible. Avoid use of metal food grinders unless previous experiment has shown that no contamination of sample with Pb or Sn results. If product to be analyzed cannot be thoroly mixed in its own container, or if composite sample of number of containers is desired, empty into large glass jar or porcelain dish and mix thoroly with wooden spoon or porcelain spatula. If liquid portion of sample cannot be incorporated into ground solid material to obtain homogeneous mixt., analyze separately. If food is packed in tins having soldered seams (sardines and meats), open tins from bottom to avoid contaminating sample with bits of solder. Avoid sifting in prepn of samples to prevent metallic contamination or segregation of Pb.

General Method

Sn and Bi Absent

(Applicable to such materials as carbohydrates, cereals and cereal products, cacao and dairy products, feeds, meats, fish, plant material, fruit and fruit products, fresh vegetables, etc., and in general to all organic materials (except fats) in which no Sn and Bi are encountered. For products containing Sn (canned foods) or Bi proceed as in **24.046–24.048**.)

24.039 REAGENTS

(**a**) *Lead std solns.*—Dissolve 20–50 g $Pb(NO_3)_2$ in min. of hot H_2O and cool with stirring. Filter crystals with suction on small büchner, redissolve, and recrystallize. Dry crystals at 100–110° to constant wt. Cool in desiccator and store in tightly stoppered bottle. (Product has no H_2O of crystn and is not appreciably hygroscopic.) Prep. stock soln contg equiv. of 2 mg Pb (3.197 mg $Pb(NO_3)_2$ /ml in 1% HNO_3, (**b**)). Prep. more dil. solns with 1% HNO_3 as needed.

(**b**) *Nitric acid.*—1%. Dil. 10 ml fresh, colorless HNO_3 (sp. gr. 1.40) to 1 L with redistd H_2O. If acid has been redistd, boil off nitrous fumes before dilg.

(**c**) *"Ash-aid" soln.*—Dissolve 40 g $Al(NO_3)_3 .9H_2O$ and 20 g $Ca(NO_3)_2.4H_2O$ in 100 ml H_2O.

(**d**) *Citric acid soln.*—Concd Pb-free soln. 1 ml = 0.5 g citric acid (reagent partially neutralized with NH_4OH during purification, **24.038**, fourth par.).

(**e**) *Diphenylthiocarbazone (dithizone).*—Dissolve ca 1 g commercial reagent in 50–75 ml $CHCl_3$ and filter if insol. material remains. Shake out in separator with four 100 ml portions metal-free (redistd) NH_4OH (1+99). (Dithizone passes into aq. phase to give orange soln.) Filter aq. exts into large separator thru cotton pledget inserted in stem of funnel. Acidify slightly with dil. HCl and ext. pptd dithizone with two or three 20 ml portions $CHCl_3$. Combine exts in separator and wash 2 or 3 times with H_2O. Draw off into beaker and evap. $CHCl_3$ with gentle heat on steam

bath, avoiding spattering as soln goes to dryness. Remove last traces of moisture by heating 1 hr at not $>50°$ *in vacuo*. Store dry reagent in dark in tightly stoppered bottle. Prep. reagent solns for extn to contain 100, 50, and 10 mg/L in freshly redistd $CHCl_3$ (*15*) and store in dark at 5–10°. (Stock soln of dithizone in $CHCl_3$ contg 1 mg/ml will keep long time and is convenient for use in making dilns.)

(**f**) *"Stripping" reagent.*—To 20 ml satd NaOAc soln add 10 ml HOAc and dil. to 100 ml.

(**g**) *Potassium iodide soln.*—2%. Prep. as frequently as necessary to prevent formation of starch-I color when mixed with reagent (**f**) in proportions specified in **24.044**(**c**).

(**h**) *Starch indicator.*—Make up 1 g sol. starch to 200 ml.

(**i**) *Sodium thiosulfate soln.*—Approx. 0.1N stock soln. Dissolve 24.8 g $Na_2S_2O_3.5H_2O$ in 1 L CO_2-free H_2O and store (preferably 2 weeks) before use. Prep. ca 0.001 and 0.005N solns by diln of the stock soln in exact ratios of 1:100 and 1:20 with CO_2-free H_2O and stdze these electrolytically, using std Pb soln equiv. to 0.2–1.0 mg Pb for the 0.001N diln and 1–5 mg Pb for 0.005N diln. Subtract anode blanks, **24.044**(**b**) and (**c**), and take as the $Na_2S_2O_3$ factor av. number of mg Pb equiv. to 1 ml of the solns. Prep. fresh dilns daily and check Pb factor at least monthly.

(**j**) *Ammonia-cyanide mixture.*—To 100 ml 10% recrystd, PO_4-free (*16*) KCN in 500 ml vol. flask add enough redistd NH_4OH to introduce 19.1 g NH_3, and dil. to vol. with redistd H_2O. (Concn of redistd NH_4OH can be detd by sp. gr. or titrn.)

(**k**) *Pure metallic tin.*—Purest obtainable, such as NBS Sample No. 42 B (0.0035% Pb). Granulate Sn as finely as possible by melting and pouring very slowly into H_2O. Det. Pb content as follows: Dissolve 1–2 g sample in HBr or HCl and volatilize the Sn by evapg soln to dryness and treating with several 5 ml portions of the HBr-Br mixt. (**l**), evapg to dryness on steam bath after each treatment. Take up with 2–3 ml HNO_3, evap. to dryness to expel Br, and take up with hot H_2O. Filter, adjust acidity to 1% with HNO_3, and proceed as in **24.044**.

(**l**) *Hydrobromic acid-bromine mixture.*—To 250 ml 40% redistd HBr add 35 ml redistd liquid Br.

(**m**) *Sodium polysulfide soln.*—Dissolve 480 g $Na_2S.9H_2O$ and 40 g NaOH in H_2O, add 16 g powd. S, shake until S dissolves, filter, and dil. to 1 L.

(**n**) *Hydrochloric-citric acid soln.*—Add quantity of reagent (**d**) equiv. to 50 g citric acid to 50 ml HCl and dil. to 250 ml.

(**o**) *Sodium oleate soln.*—10%. To 45 ml 30% NaOH soln and 400 ml H_2O in 1.5 L beaker, add slowly, while heating and stirring, 90 g (by difference from separator) oleic acid. Heat mixt. on steam bath until soap is entirely dissolved. (Small flocculent ppt of impurities may remain.) Cool, dil. to 1 L, mix, and filter.

(**p**) *Ammonia-cyanide-citrate soln.*—Dissolve 10 g PO_4-free KCN and 10 g citric acid in 250 ml NH_4OH (sp. gr. 0.90) and dil. to 1 L. Reagent is conveniently preserved in dispensing app. that causes min. volatilization of NH_3.

24.040 PREPARATION OF SAMPLE (ASHING)

Quantity of material taken for sample depends upon amount available and expected Pb content, and whether Pb is to be detd as in **24.044** or **24.045**. In general, weigh representative sample of 5–200 g, depending upon conditions, into suitable porcelain dish or casserole. Dry wet samples on steam bath or in oven. Add 2–5 ml of the "ash-aid" soln, **24.039**(**c**), to products difficult to ash (meats), or to furnish ash bulk to low ash products (candies, and jellies low in fruit content); mix well, and dry.

Char gelatin, carbohydrate foods such as jam, and other products that tend to swell excessively, by carefully heating over burner. (Swelling can be controlled by playing small flame from glass jet over surface of material in dish, but metallic burner must not be used for this purpose because of possible metallic contamination.) Do not let material ignite. Milk, candies, etc., may be charred without ignition by adding sample little at time to casserole heated over burner or hot plate. (Overhead radiant heater is often very convenient.) When samples are dry or charred, place in temp.-controlled muffle and raise temp. *slowly* to 500° without ignition.

If sample contains fat, "smoke" it away by heating long enough at ca 350°. Cover floor of muffle with piece of asbestos board or SiO_2 plate so that sample receives most of its heat by radiation from sides and roof and not by conduction from hotter floor of muffle.

If muffle has automatic control, ash overnight at not $>500°$. If sample is not completely ashed next morning or if day-time ashings at 500° are not proceeding satisfactorily, remove casserole, cool, and moisten char with 2–5 ml of the ash-aid. Dry contents of casserole past danger of spattering (no free liquid) and replace in muffle. If ashing is not complete or proceeding rapidly after 30 min., remove casserole, cool, and cautiously add 2–3 ml HNO_3. Dry, place in muffle, and continue ashing until practically C-free. Avoid excessive use of ash-aid, and particularly HNO_3, if sample still contains much intermixed C, because local overheating or deflagration may result, especially if much K is present in ash.

When clean ash is obtained, cool, cover casserole with watch glass, and add cautiously 15–20

ml HCl. Rinse down watch glass with H_2O and heat on steam bath. If *clear* soln is not obtained, evap. again to dryness and repeat addn of HCl. If insol. matter persists, evap. HCl and dehydrate SiO_2 by heating to fumes with 5–10 ml 60% $HClO_4$ (double distd preferred). If $HClO_4$ is used, considerable H_2O (200 ml) may be necessary to completely dissolve $KClO_4$ later as when KCN is used in dithizone extn of Pb, **24.042.**

Dil. with H_2O and filter soln when necessary with suction thru fine fritted glass filter. Catch filtrate in 500 ml g-s. erlenmeyer under bell jar. Leach insol. material on filter successively with few ml hot HCl, the hot HCl-citric acid soln, and hot 40% NH_4OAc soln.

In certain instances take following special precautions:

(1) If quantity of insol. material (SiO_2) remaining on filter is abnormal, flush it into Pt dish with H_2O, evap., and treat residue with one or two 5 ml portions HF. Evap. to dryness, take up residue with H_2O and few drops of HCl or $HClO_4$, and add to bulk of ash filtrate.

(2) When ashing is of long duration, no ash-aid has been used, or natural ash is low with little ash bulk, Pb may be baked on dish. To remove this Pb, add few pellets (2–3 g) of NaOH and dissolve in few ml hot H_2O. Tilt dish so that sirupy soln completely wets that portion of interior originally occupied by sample; then heat short time on steam bath, but do not bring to dryness. (Overheating with concd NaOH may result in extg few mmg Pb from casserole. Porcelain retains Pb to less extent than does SiO_2 but may contain very small quantities of Pb.) Take up residue with H_2O and add directly to filtrate. Finally rinse dish with few ml hot HCl followed by hot H_2O.

24.041 ISOLATION OF LEAD

Method **24.042,** while rapid and convenient, is limited to those materials that, with aid of citric acid, yield clear ammoniacal soln demanded for quant. extn of Pb with dithizone. Pb is readily occluded by many alk. ppts (Mg and Ca phosphates, Al and Fe hydroxides and silicates). Many food materials may be handled in this way because the naturally occurring quantities of these substances are not excessive. However, some materials contain more of these substances than can be kept in soln under alk. conditions with any reasonable quantity of citric acid (*17*). In these cases proceed as in **24.043.** Difficulty of ammoniacal pptn may sometimes be overcome by limiting sample size in cases where sampling is no problem.

24.042 *Dithizone Extraction*

(Applicable to most carbohydrates and cereal foods, fruit and fruit products, milk, fresh vegetables, plant materials, etc.)

Transfer ash soln to 300 ml short-stem separator and add citric acid reagent, **24.039(d)**, equiv. to 10 g citric acid. Make slightly alk. to litmus with NH_4OH, keeping soln cool, and let stand 1–2 min. If ppt forms, redissolve with HCl and isolate the Pb as in **24.043.** If no ppt forms, add 5 ml 10% KCN soln (more may be necessary if large quantities of Zn, Cu, Cd, etc., are present) and check pH of soln by adding drop of *thymol blue soln* and observing color of drop (pH should be 8.5 or above, blue-green to blue with thymol blue).

If ash was highly colored with Fe, keep pH of soln comparatively low, because pH of 10 or above in presence of Fe may cause oxidation of dithizone. Immediately ext. with 20 ml portions of the dithizone reagent, using the more dil. solns unless exceptionally large quantities of Pb are present. Shake 20–30 sec., let layers sep., and note color of $CHCl_3$ phase. (Pb dithizone complex is red, but color may be masked by excess green dithizone, giving intermediate hues of purple and crimson. Color of $CHCl_3$ ext. gives first indication of quantity of Pb present, and progress of extn can be followed by noting color of successive exts.)

(**a**) *If Pb is to be determined electrolytically* (Pb $>$ 0.05 mg), drain $CHCl_3$ layer into 125 ml short-stem separator contg 25–30 ml H_2O made ammoniacal with *one drop* NH_4OH (sp. gr. 0.90). Continue extn until 2 successive exts with small portions of the more dil. dithizone solns show the negative green (not bluish or purple) color, combining exts in smaller separator. Shake, let layers sep., drain $CHCl_3$ fraction into another small separator, and repeat washing process as before. Drain $CHCl_3$ fraction as cleanly as possible into 100 or 150 ml beaker, and pass small portion of dil. dithizone soln thru separators in succession so as to wash out small portions of ext. persisting in aq. fraction. Add to beaker and evap. $CHCl_3$ with gentle heat on steam bath. Take up dry residue with 3–4 ml HNO_3, and heat by swirling over low flame. Dil. to ca 25 ml and continue heating 1–2 min. in order to expel oxides of N. Add small piece of litmus paper, neutralize with NH_4OH, dil. nearly to capacity of beaker, and add 1 ml colorless HNO_3/100 ml soln. Proceed as in **24.044(b)** and (**c**).

Alternative method.—Drain washed $CHCl_3$ into separator contg 110 ml 1% HNO_3, **24.039(b)**. Shake vigorously 1 min. to decompose dithizonate and drain off green $CHCl_3$ soln. Filter acid soln thru dry filter and pipet 100 ml aliquot into 150 ml beaker. Proceed immediately as in **24.044(b)** and (**c**), taking care to heat and stir soln to volatilize dissolved $CHCl_3$ before adding $K_2Cr_2O_7$ and closing electrolytic circuit. Multiply results by factor 1.1 (*18*).

(**b**) *If Pb is to be determined by colorimetric dithizone method* (Pb < 0.2 mg), do not wash dithizone exts with the dil. NH_4OH, but drain directly into smaller separator contg 25 ml of the 1% HNO_3, **24.039**(**b**). When extn is complete, shake combined exts in smaller separator and drain green dithizone layer into another separator contg addnl 25 ml portion 1% HNO_3. Shake, let layers sep., and discard $CHCl_3$ fraction. Filter acid exts contg Pb in succession thru small pledget of wet cotton inserted in stem of small funnel, into 50 ml flask or g-s. cylinder, using second acid ext. to wash out separator in which first acid extn was made. (This procedure removes $CHCl_3$ globules.) Make up any slight deficiency in vol. with the 1% HNO_3 and mix. Proceed as in **24.045.**

24.043 Sulfide Separation

(Applicable to all products and usually necessary in case of cacao products, tea, sardines, and all food products contg high proportion of alk. earth phosphates, especially those of Mg, which promote formation of ppts in ammoniacal citrate solns.)

Cool acid soln of ash, add citric acid soln, **24.039**(**d**), equiv. to 10 g citric acid, and adjust to pH 3.0–3.4 (bromophenol blue) with NH_4OH. If enough Fe is present to color soln strongly, make final adjustment with help of spot plate. (Phosphates pptd by local action of NH_4OH may usually be redissolved by shaking and cooling.) If quantity of Pb is small, add 5–10 mg pure $CuSO_4.5H_2O$ to soln to act as coprecipitant. Ppt sulfides by passing in H_2S until soln is satd (3–5 min.). Immediately filter with suction into flask in bell jar (fine fritted glass filter is preferred).

(**a**) *If Pb is to be determined electrolytically* (Pb > 0.05 mg) wash flask and ppt with few small portions of 3% Na_2SO_4 adjusted to pH 3.0–3.4 and satd with H_2S. If clean sulfide ppt has been obtained, dissolve sulfides with 5 ml hot HNO_3, wetting all portions of filter; let stand few min. and draw thru into flask in which sulfide pptn was made. Wash filter with several portions of hot H_2O, stopper flask, shake, and boil few min. to remove traces of H_2S. Cool, adjust acidity to 1% with HNO_3 in 100–125 ml vol., and proceed as in **24.044**(**b**) and (**c**). If there is possibility of sulfide ppt being contaminated with >3 mg Cl, 20 mg As_2O_3, 30 mg P_2O_5, 50 mg Hg, or with Sb_2S_3, dissolve as above with HNO_3 (without previous washing with Na_2SO_4 soln), wash filter with hot H_2O, and boil soln as before. Transfer to 200 ml separator, add citric acid soln equiv. to 5 g citric acid, make ammoniacal, ext. with dithizone soln, and det. Pb as in **24.042, 24.042**(**a**), and **24.044**(**b**) and (**c**).

(**b**) *If Pb is to be determined by colorimetric dithizone method* (Pb < 0.2 mg), dissolve sulfides, without previous washing, with 5 ml hot HNO_3, drawing soln thru into original flask; wash with hot H_2O, stopper, shake, and boil to remove H_2S. Transfer to 200 ml separator, add citric acid soln equiv. to 5 g citric acid, make ammoniacal, ext., and det. Pb as in **24.042, 24.042**(**b**), and **24.045**(**a**) or (**b**).

DETERMINATION OF LEAD

24.044 *Electrolytic Method*
(Pb 0.05–10.00 mg)

(**a**) *Apparatus.—See* Fig. 45. Four dry cells in series constitute convenient source of current. Meter (0–500 milliamperes), switch, fuse, rheostat (60 ohm radio type), and variable resistance for control of motor speed may be conveniently mounted upon panel. Motor for rotating anode (1/20 H.P., 110 V. universal) is equipped with chuck and binding post. Rate of rotation should be sufficient to produce efficient circulation and may vary from 400 to 800 rpm. Electrodes consist of 45-mesh, sand-blasted, Pt gauze cylindrical anode, $1 \times 5/16''$ with 3″ stem, and cathode of 18-gauge Pt wire wound spirally. For larger quantities of Pb (>5 mg), cylindrical anode $2 \times \frac{1}{2}''$ is convenient. (Commercial app. equiv. to above is available.)

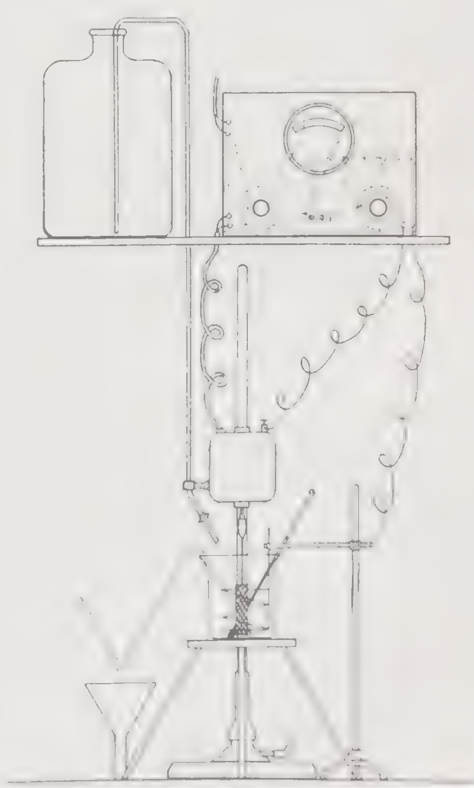

FIG. 45.—APPARATUS FOR DETERMINATION OF LEAD BY ELECTROLYTIC METHOD

(b) *Electrolysis.*—Immediately before electrolyzing bring anode to red heat in oxidizing flame of burner. (Variable titrn blank is obtained if anode is not heated just before detn, due possibly to film of O adsorbed on anode and activated during electrolysis. Heating reduces and renders constant this "O blank." With small anode it will be 0.07–0.1 ml 0.001N $Na_2S_2O_3$ and with larger electrode proportionately larger. Blank for each anode should be detd from av. of series of detns conducted on pure reagents.)

In all detns, sample at this point is contained in 100–125 ml 1% HNO_3 (with large anode, vol. of 200 ml is convenient). Place beaker (100–150 ml for small and 250 ml for large anode) in position, making sure electrodes are well covered with soln, and start motor. Heat to 60–70°; then add ca 100 mg $K_2Cr_2O_7$ to keep soln in oxidized state and repress formation of nitrites, especially when org. matter is present. Start current and electrolyze with ca 75 milliamperes 20 min. at 70–80°. Use 100–150 milliamperes for larger anode. Remove flame, insert siphon in beaker, and start stream of H_2O playing directly on anode. Start siphon, taking care to keep level of liquid above deposit. (Convenient siphon can also be made by connecting inverted V-shape tube to ordinary H_2O pump.) Acid is entirely removed when current falls to zero. Turn off motor, electrolytic current, and rinse H_2O; remove anode from chuck and give final rinse with H_2O.

(c) *Titration.*—Dissolve deposit in 4–5 ml of the "stripping" reagent, **24.039(f)**, +1 ml of the KI reagent, **24.039**(g), contained in flat-bottom vial of such size that soln just covers anode. Add few drops of the starch soln, **24.039(h)**, and titr. liberated I with 0.001N $Na_2S_2O_3$, **24.039(i)**, in the vial, using anode as stirrer and sighting down thru vial, as thru miniature Nessler tube, to detect the delicate end point. (If quantity of Pb is seen to be large (1–5 mg), use 0.005N $Na_2S_2O_3$ and double amount of reagents **24.039(f)** and (**g**). With 2″ anode still larger amounts may be used.) No yellow insol. PbI_2 should form as deposit is "stripped"; if it does, add more of the NaOAc soln. Deposit should dissolve completely and almost immediately. To det. quantity of Pb, subtract anode and reagent blanks from total titer and multiply by factor of the $Na_2S_2O_3$ soln, **24.039(i)**.

$$PbO_2+4HI=I_2+PbI_2+2H_2O.$$

Absence of interfering Bi may be assured by applying test **24.048(c)**.

24.045 *Colorimetric Dithizone Method* (*19*)
(Pb 0.001–0.200 mg)

Limiting factor in detn of minute quantities of Pb by colorimetric dithizone method is probably size of reagent blank. Importance of careful blank detns must be especially stressed when quantities of Pb of order of 1–5 mmg are being detd. With special care in purification of reagents and by use of carefully cleaned Pyrex ware, including separators, it is possible to reduce reagent blank to 1 mmg or less. Owing to Pb-bearing dust, vapors, etc., it is necessary to expose blank detn in muffle or on steam bath for same length of time as sample is exposed, and to use exactly same quantities of reagents (even H_2O) for blank and actual detns.

Pb is extd from aq. soln, under std conditions of vol. and pH, with definite vol. of $CHCl_3$ soln of dithizone of std concn. Optimum pH of operation is 9.5–10.0. Dithizone strengths are so chosen that excess dithizone is always present in reaction mixt. Pb is brought into $CHCl_3$ phase in form of red complex, and uncombined green dithizone partitions between aq. and $CHCl_3$ phases and modifies color of ext. according to relative quantities of Pb and dithizone. Thus, according to this proportion, series of colors from red to green may be arranged with intermediate crimsons, purples, and blues. Vols and strengths of the $CHCl_3$ solns depend upon Pb range it is desired to cover and are so chosen as to give same general color progression from red to green for each range. Limiting range increases accuracy at expense of flexibility. Colors produced with std quantities of Pb furnish basis for quant. estimation by comparison. Vols and concns of std dithizone for various ranges are as follows when 10 mm cell is used:

Pb RANGES *mmg (0.001 mg)*	CONCN *mg/L*	VOLUME *ml*
0–10	8	5
0–50	10	25
0–200	20	40

See Snyder, Anal. Chem. **19**, **684**(1947), for modification operated at pH 11.5.

(a) *Simple color matching.*—Prep. 10 stds covering in equal steps the desired concn range, as follows: Use std Pb soln, **24.039(a)**, in 1% HNO_3, 1 ml of which equals some simple fraction or multiple of 1 mmg Pb. Measure quantities representing various steps of range into series of separators and add the 1% HNO_3 so that total vol. is always 50 ml. (Add acid first so that Pb soln is not lost around stopcock of separator.) Add 10 ml NH_3-cyanide mixt., **24.039(j)**, and mix. Resultant pH will be ca 9.7. Immediately add appropriate vol. std dithizone soln, which depends on range to be covered (*see* table), and shake 1 min. Drain lower layers into series of tubes or vials and arrange in order. For lower ranges, i.e. up to 20 mmg Pb, matching is best done by viewing longitudinally in small flat-bottom vials ca 3″ long. For higher ranges, 0–50 mmg and above, depth of column must be reduced, and matching is conveniently done by viewing trans-

versely in Nessler tubes of matched diam., because even pure dithizone solns appear red by transmitted light if concn or depth of column is increased beyond certain point. If stds are kept covered when not in use they should last at least 1 day.

For the detn, place aliquot part, or entire amount, of the 50 ml 1% HNO_3 in which Pb has been isolated, **24.042(b)** or **24.043(b)**, in separator, and if aliquot is taken, dil. to 50 ml with 1% HNO_3. Add 10 ml NH_3-cyanide mixt., **24.039(j)**, and mix. Immediately develop color by shaking 1 min. with proper quantity std dithizone soln. Drain lower layer into tube or vial similar to those used with stds and compare. If range is exceeded, repeat with smaller aliquot or re-ext. with excess dithizone before draining from separator, isolate once more in 50 ml 1% HNO_3 reagent, and compare with stds covering higher range. Interpolation between steps of various ranges should be easily made.

If aliquot of the 50 ml 1% HNO_3 in which the Pb has been isolated is taken, subtract only corresponding quantity of total reagent blank from quantity of Pb found.

(**b**) *Photometric methods.*—Absorption spectra of the 2 components in dithizone ext. (Pb dithizone complex and free dithizone) show marked difference in their ability to absorb light of wavelength 510 mμ, red Pb complex absorbing strongly and green dithizone transmitting freely. Thus, when absorption of light of this wavelength by individuals of std color series, measured thru suitable cell length, is detd photometrically, linear relationship is observed between quantities of Pb and absorbance. In making measurements, spectrophotometer set at this wavelength or photometer equipped with blue-green filter centered at about this point can be used. Dithizone solns are stdzd once only with known quantities of Pb, and labor of repeated std prepn is unnecessary.

Stdze dithizone solns as follows: Using appropriate vols and concns of solns specified for various ranges (*see* above) in separators, prep. std colors as in visual color-matching procedure, satg the std Pb and the 1% HNO_3 solns with clear $CHCl_3$ before use, and thereby eliminating differences in vol. of ext. between stds and unknowns. (It is unnecessary to prep. full 10 steps of the range, and number of stds may be limited to 5 or 6.) Develop colors by shaking separators 1 min., let stand few min., and filter exts thru specially prepd papers (9 cm quant. papers soaked overnight in 1% HNO_3 and washed with large vols H_2O on büchner to remove slight trace of acid and/or Pb usually present on even best grades of filter paper. Fitting 9 cm paper directly into mouth of 50 ml Pyrex beaker eliminates need of funnel in filtering operation.) Fill cell with filtered exts and det. absorbances for various steps of range.

Plot against quantity of Pb to obtain stdzn curve for particular lot of dithizone. Preferably calc. slope of line connecting std points and intercept of line on Pb axis, making calcn by least squares method as follows: Take equation of line connecting std points as $Y = a + bX$, and let X = mmg Pb and Y = absorbance; a then represents intercept on Pb axis (in this case negative value) and b represents tangent or slope of line. Calc. a and b from following formula, where n = number of observations, including that for 0 Pb, and Σ represents merely "sum":

$$b = \frac{\Sigma XY - \dfrac{\Sigma X \Sigma Y}{n}}{\Sigma Y^2 - \dfrac{\Sigma Y \Sigma Y}{n}}, \quad \text{and} \quad a = \frac{\Sigma X}{n} - b\,\frac{\Sigma Y}{n}$$

Then procedure for detg Pb content of unknown falling within the range is to det. absorbance, using std dithizone and same cell with which std readings were made, and to calc. Pb from equation $Y = a + bX$, using values of a and b detd previously. If protected from evapn and direct sunlight, std factors of dithizone solns should not change appreciably for at least one month (*15*).

For actual detn proceed as in (a), except to filter ext. thru the prepd papers before photometric measurement. Det. absorbance, using the stdzd dithizone with same cell used in making std curve, and read quantity of Pb from this std curve or calc. from factor of dithizone soln. If range is exceeded, repeat with smaller aliquot, or re-ext. and repeat with dithizone stdzd to cover higher range. If aliquot of the 50 ml 1% HNO_3 in which Pb has been isolated is taken, subtract only corresponding quantity of total reagent blank from quantity of Pb found.

24.046 INTERFERENCES

If present in excessive quantities in final detn, Cl, P_2O_5, As, Se, Te, Hg, and Bi (>5 mg) will prevent complete electrolytic deposition of Pb; and Bi (<2 mg), Sn, Sb, Mn, and Ag will contaminate deposit. Certain reducing agents, such as nitrites, likewise prevent complete deposition of Pb. General method leading up to final detn of Pb by electrolytic method has been so formulated that all interferences except those of Sn and Bi are eliminated. Special directions, applicable to both electrolytic and colorimetric methods, for removal of Sn and Bi are given in **24.047** and **24.048**. As much as 3 mg Cl, 20 mg As_2O_3, 30 mg P_2O_5, and 50 mg Hg will not interfere in final electrolysis, and if there is suspicion that greater quantities are present in sulfide mixt., **24.043**,

they can be eliminated by dithizone extn. Interferences in colorimetric dithizone method are limited by use of KCN to stannous Sn, Bi, and Tl. Rarity of Tl makes its interference unlikely in ordinary work, and no method of removal is given (*17*). Dithizone itself is destroyed by strong oxidizing agents, such as free halogens and large quantities of ferric Fe, under conditions of dithizone extn of Pb.

24.047 Removal of Tin

Sn becomes problem in analysis of canned foods, and in quantities >150 ppm it will usually appear in ash soln as milky suspension of SnO_2. It must be dissolved to facilitate filtration and to release occluded Pb. Quantities of Sn of this order may cause trouble by pptg under conditions of dithizone extn of Pb, **24.042.**

Two procedures for elimination of larger quantities of Sn are given: (**a**) Volatilization as $SnBr_4$ from acid soln of ash, and (**b**) leaching mixed sulfides with warm Na polysulfide soln, when sulfide method of isolation, **24.043,** has been applied. These procedures may not eliminate Sn completely, but quantity should be reduced to below that necessary to interfere with electrolytic detn of Pb. Stannic Sn is not extd with dithizone, and as small quantities of residual Sn will be in stannic form after application of either (**a**) or (**b**), final isolation of Pb by dithizone extn will eliminate Sn completely.

In general, quantities of <100 mg should not interfere in either electrolytic or colorimetric dithizone methods of Pb detn provided Sn is in stannic form and preliminary isolation with dithizone is made; hence, this method of isolation should be applied wherever possible.

(**a**) *Volatilization as $SnBr_4$ from acid soln of ash.*—After almost C-free ash is obtained, **24.040,** add 15–20 ml 40% redistd HBr. If nitrates were used as ash aids, cover casserole with watch glass and heat on steam bath until Br evolution diminishes; then rinse off watch glass with H_2O and bring to boil to complete expulsion of Br. (This process destroys undecomposed nitrates.) Add more HBr if necessary to dissolve ash, and examine solns for clearness. If there is insol. residue of SnO_2, add 50–100 mg pure Sn, **24.039**(**k**), to simmering HBr soln of ash and let it dissolve. (Metallic Sn is best agent to bring ignited SnO_2 into soln. To be effective, ash soln must be in reduced state. Fe_2O_3 sometimes becomes "noble" during ashing and dissolves with difficulty, but treatment with metallic Sn also brings it into soln. Treatment with Sn is necessary only with contents of badly corroded cans.)

When soln of ash is free from milkiness due to SnO_2, add 20 ml 60% $HClO_4$ (double distd preferred), oxidize mixt. with few ml HBr-Br mixt., **24.039**(**l**), and then add addnl 15 ml of the reagent portionwise, while soln is evapd to incipient fumes of $HClO_4$ (ca 150°) on hot plate. Repeat with another 10 ml portion HBr-Br mixt. if >100 mg Sn was used to dissolve ash. (Hot $HClO_4$ helps keep ash salts in soln and with Br holds the Sn as volatile $SnBr_4$.) When the HBr and Br are completely volatilized, cool, and take up with hot H_2O (200 ml may be necessary if much $KClO_4$ is present). Filter off any small quantities of dehydrated SiO_2, ext. residue twice with 5 ml hot HCl-citric acid reagent, **24.039**(**n**), and hot H_2O, treat dish if necessary with NaOH as in **24.040**(2), and isolate Pb by dithizone extn as in **24.042,** or by sulfide sepn, **24.043,** finally detg Pb as in **24.044**(**b**) and (**c**) or **24.045**(**a**) or (**b**).

(**b**) *With sodium polysulfide.*—(Recommended for routine work on canned foods by electrolytic method when Pb>0.05 mg.)

Isolate Pb by sulfide pptn, **24.043,** filter, and wash flask and filter with 3–6 portions of ca 5 ml each of warm Na polysulfide soln, **24.039**(**m**). (Sn, As, and Sb sulfides are dissolved; CuS may be partially dissolved and repptd in filtrate.) Wash flask and residual sulfides several times with 3% Na_2SO_4 soln adjusted to pH 3.0–3.4 and satd with H_2S, and proceed as in **24.043**(**a**), beginning "dissolve sulfides with 5 ml hot HNO_3 . . . " and continuing directly to electrolytic detn, **24.044**(**b**) and (**c**). When ash contains much Sn, as when metallic Sn has been added to dissolve insol. metallic oxides, sulfide ppt will be so bulky as to be difficult to handle, and it will be necessary to use volatilization method (**a**) before sulfiding. For colorimetric dithizone detn of Pb, ext. HNO_3 soln of dissolved sulfides and proceed as in **24.043**(**b**) and **24.045**(**a**) or (**b**).

24.048 Detection and Removal of Bismuth

(**a**) *By dithizone at pH 2.0 after preliminary dithizone extraction at pH 8–11* (*20*).—(This procedure completely removes small quantities of Bi.)

Ext. metals from the $CHCl_3$ dithizone ext. with 50 ml 1% HNO_3 as in **24.042**(**b**). Adjust acid ext. to pH 2.0 (metacresol purple indicator) with 5% NH_4OH soln and shake vigorously ca 1 min. with 10 ml $CHCl_3$ soln of dithizone (200–250 mg/L). Let layers sep., and if $CHCl_3$ ext. is orange red to red (Bi), drain off and ext. with another 10 ml portion of the dithizone soln. If shades of green or purple are visible, indicating excess dithizone, drain $CHCl_3$ ext. and ext. aq. phase once more with 5 ml of the dithizone soln (shaking should be prolonged, 3–5 min., to insure complete extn of Bi). Continue extns until dithizone ext. remains pure green. Adjust aq. soln to pH 8.5 with NH_4OH, add KCN, and ext. with dithizone as in **24.042.** Det. Pb colorimetrically as in **24.042**(**b**)

and **24.045**(a) or (b), or electrolytically, **24.042**(a) and **24.044**(b) and (c), when Pb > 0.05 mg.

(Procedure of Bambach and Burkey (*21*) seps small quantities of Bi from Pb by shaking out $CHCl_3$ soln of their mixed dithizonates with aq. soln buffered at pH 3.4; Bi remains as the dithizonate in $CHCl_3$ phase, while Pb enters aq. phase and can be sepd Bi-free. Only *slight* excess of free dithizone should be present in $CHCl_3$ mixt. of dithizonates, otherwise Pb does not strip out completely. System of photometric detection and evaluation of Bi interference has also been outlined (*17*).)

(b) *From acid soln of sulfides.*—(Intended for small quantities of Bi, particularly when sulfide sepns may be necessary.) Dissolve mixed sulfides, **24.043,** with hot HNO_3 and sep. Bi and Pb as in (a).

Special conditions.—(Intended for products contg large quantities of Bi.) Dissolve inorg. Bi compounds directly in HBr-Br, **24.039**(l). Prep. org. Bi compounds or Bi prepns mixed with org. matter contg little ash, as in **24.040,** and dissolve residue in HBr-Br. If sample contains org. matter with appreciable ash material other than Bi compounds, proceed as in **24.040** or **24.050,** apply sulfide sepn, **24.043,** and dissolve mixed sulfides in HNO_3. Evap. HNO_3 soln of sulfides to dryness in porcelain dish and treat with small portions of the HBr-Br mixt. Evap. contents of dish contg Bi dissolved in HBr-Br, after any of above methods of prepn, on steam bath to volatilize Sn and to convert other metals to bromides. Evap. to dryness, place in muffle with temp. control, and raise temp. gradually to 300°. ($AsBr_3$ and $SbBr_3$ volatilize first at 100° or above; $BiBr_3$ volatilizes as dense orange fumes at 300°.) After 5 min., or when fumes cease, remove dish, cool and treat again with small portions of HBr-Br. Again evap. to dryness and heat addnl 5 min. at 300–325° ($PbBr_2$ does not volatilize appreciably at <350°). Remove dish, cool, and dissolve residue in hot HNO_3. Proceed with removal of last traces of Bi at pH 2.0 and det. Pb as in (a).

(c) *After PbO_2 titration in electrolytic method.*—Add to soln from **24.044**(c) in titrg vial 0.25 g solid KI and ca 0.5 ml HCl. Shake, and add just enough dil. $Na_2S_2O_3$ soln to discharge any starch-I color. Pure yellow color shows presence of the double Bi iodide. (Under conditions of test, there is no interfering Cu, ferric Fe, or Sb, and 0.005 mg Bi shows yellow color test.) If test is positive, reject Pb results and repeat detn, giving special attention to removal of Bi interference.

Special Methods of Sample Preparation

24.049 Solution in Acids

(Applicable to chemicals soluble in water or acid, e.g., phosphates, sulfates, etc., and org. products of type of tartrates and citrates)

Dissolve 5–100 g sample, according to its nature and quantity of Pb expected, in HCl in 400 ml beaker. With Ca phosphates use 10–50 g. Dissolve in smallest practicable vol. of soln by warming and adding alternately small quantities of hot H_2O and HCl. Filter soln with suction (fritted glass preferred) into beaker or flask under bell jar and leach any residue with 10–25 ml hot HCl-citric acid, **24.039**(n), followed by 10–25 ml hot 40% NH_4OAc soln. Rinse beaker and filter with hot H_2O and cool soln.

Proceed as in **24.042.** If interfering ppt forms, again acidify and isolate Pb by sulfide pptn, **24.043.** If it is difficult to obtain clear soln with Ca phosphates at pH 3.0–3.4 (sulfide ppt may be contaminated with excessive phosphates), redissolve ppt, add more citric acid soln, **24.039**(d), readjust pH, and reppt sulfides; or make one sulfide pptn, dissolve sulfides in hot HNO_3, boil off H_2S, and ext. Pb with dithizone, **24.042.** Sometimes difficulty due to ppt formation in **24.042** can be avoided by using smaller sample for extn and colorimetric detn. If Sn or Bi is suspected, remove by methods described in **24.047** and **24.048.** Finally det. isolated Pb electrolytically, **24.044,** or colorimetrically, **24.045.**

24.050 Complete Digestion

(Applicable to most food or biological products; with difficulty to fats and oils, oily products, etc.)

Digest representative sample in Kjeldahl flask as in **24.003.** Distill As, if desired, according to bromate method, **24.009.** If As is not to be distd, add 100 ml H_2O and enough HCl to flask to dissolve any $CaSO_4$ in residue. Filter on fritted glass filter, pulverizing any insol. residue (anhyd. SiO_2 or $BaSO_4$) with flat-end stirring rod. Dissolve any $PbSO_4$ in flask and leach residue on filter with 10–20 ml hot HCl-citric acid soln, **24.039**(n), followed by 10–20 ml hot 40% NH_4OAc soln. Finally rinse both flask and filter with hot H_2O. Isolate Pb by dithizone, **24.042,** or sulfide pptn, **24.043,** methods. (In general, sulfide method is preferable, especially when $BaSO_4$ or excessive $CaSO_4$ is present, as insol. sulfates readily occlude Pb.) If Bi and Sn are present, remove them as in **24.047** and **24.048.** After isolation, det. Pb according to electrolytic, **24.044,** or colorimetric method, **24.045.**

24.051 Partial Digestion or "Mush"

(a) *For fruits or vegetables that can be peeled.*—Weigh and peel representative sample (10–45 apples), including, if desired, stem and calyx ends with peels. Transfer peels to one or more 2 L tared beakers, reweigh, and record wt peels. Add 75–200 ml HNO_3 to each beaker, according to wt peel taken, and warm carefully over gauze or on

steam bath in fume hood. Stew slowly, while stirring, until initial foaming decreases. Cover beaker with watch glass and continue heating until mixt. is smooth with little or no stringiness and greatly diminished evolution of oxides of N (15–45 min. according to sample taken). Pectin must be sufficiently destroyed to prevent emulsification in subsequent $CHCl_3$ extns. Dil. with H_2O, cool, and transfer contents of the one or more beakers to 1 or 2 L vol. flask. Dil. to mark, mix well, and filter.

Transfer 100–250 ml filtrate to short-stem separator, add citric acid soln, **24.039**(**d**), equiv. to 5 g citric acid, make ammoniacal (soln will darken materially), and proceed with dithizone extn as in **24.042.** (If soln contains much sugar, extra cyanide may be necessary and Pb should be extd immediately. Sugar residues combine with cyanides and weaken or completely destroy "masking" effect of cyanide. If cyanide is combined in ineffective combinations, other metals, notably Zn, may be extd.) Det. extd Pb electrolytically, **24.042**(**a**) and **24.044**(**b**) and (**c**). Correct for vol. occupied by insol. matter by allowing 0.075 ml/g peel.

(**b**) *For products other than fruit and vegetable peels.*—(Carbohydrate foods, fresh or canned small fruits or vegetables, jams, apple butter, etc. Sn is often present, while Bi is usually absent.)

Weigh 100–200 g well-mixed sample into 1 or 2 L beaker. To dry samples add ca equal wt H_2O and 50–150 ml HNO_3, and "mush" mixt. as in (**a**). (Vary time of mushing and quantity of HNO_3 according to product. Colloids, which induce emulsification in dithizone extn, should be destroyed so that clear soln is obtained upon filtration.) Cool, transfer to 500 ml vol. flask, dil. to mark, mix well, and filter. Transfer 100–250 ml aliquot of filtrate to separator and proceed as in (**a**), concluding with electrolytic detn. (Interference of Sn is generally negligible.)

Rapid Method Restricted to Apples and Pears

(Efficiency of 95% expected)

(For rapid detn of Pb spray residue on apples and pears; ppm $\times$0.007 = grains/lb; (grains/lb) $\times$143 = ppm)

24.052 PREPARATION OF SAMPLE

Weigh 10 or more apples or pears and pull or cut out stems with narrow-blade knife, cutting no more of flesh than necessary. Trim off sepals (dried residue of blossom) and discard sepals and stems. To 25 ml 30% NaOH soln in 600 ml beaker, add 175 ml H_2O and 25 ml Na oleate soln, **24.039**(**o**), and bring to gentle boil. Have ready in wash bottle 250 ml hot HNO_3 (2+98) or hot HCl (3+97). (Reasonably accurate figure for As_2O_3 can be obtained by using the HCl rinse and applying Gutzeit As detn, **24.005**, to portion of filtrate, after acidifying part of the 500 ml alk. strip soln with $\frac{1}{10}$ vol. HCl instead of HNO_3 (*see* later in this section). Rapid method for F, **24.035**, likewise specifies HCl rinse and acidification.)

Impale each fruit in turn upon pointed glass rod; immerse in the alk. soln, with occasional rotation, until skin begins to check; then remove to large funnel inserted in 500 ml vol. flask and rinse with stream of the hot acid, being careful to flush out stem and calyx ends thoroly. When all fruit has been thus treated, cool alk. soln and add it thru funnel to acid soln in flask. Rinse beaker and funnel with any remaining acid and with H_2O, using entire 250 ml rinse acid. Cool, and dil. to vol.

In dry 200 ml erlenmeyer place exactly 10 ml HNO_3 (10 ml HCl for As or F). Thoroly mix contents of vol. flask and immediately add 100 ml to acid in erlenmeyer while swirling vigorously. Filter on rapid paper. If first portion of filtrate is cloudy, refilter until clear. Det. Pb as in **24.053** or **24.054,** or use 25 ml acid and 250 ml wash soln and proceed electrolytically as in **24.055.** (*See* Fahey, Cassil, and Rusk (*2*) for details of churn-type washer for removing Pb spray residues from apples and pears.)

24.053 DETERMINATION WITH NESSLER TUBES

(At least 15 tall form tubes matched for uniformity in color *and diam.* are necessary.)

(**a**) *Standards.*—Add to each of two 1 L vol. flasks 47.5 ml 30% NaOH soln. If HNO_3 was used in rinsing and acidification, **24.052,** add 100 ml HNO_3 to each flask. If HCl (3+97) was used in rinsing, add to each flask 91 ml HNO_3 and 13.6 ml HCl. Do not mix in the acids unless solns are cold and dil. To one of flasks add stock reagent, **24.039**(**a**), equiv. to 25.45 mg Pb. Mark this flask "std" and other "blank." Dil. both solns to vol. at room temp. and mix. These 2 solns contain reagents as they occur in acidified and filtered sample soln. The "std" is equiv. in Pb content to acidified soln from sample of 1400 g carrying Pb load (removable by "stripping" procedure) of 10 ppm. By combination of the 2 solns in suitable proportions, equiv. of any Pb load from 0 to 10 ppm may be obtained.

Std tubes made up in intervals corresponding to 1.0 ppm may be interpolated to 0.5 ppm. Following table gives quantities of "std" and "blank" to be added to Nessler tubes for each interval; they are conveniently measured into tube by burets:

Pb ppm	"standard" ml	"blank" ml
0.0	0.0	10.0
1.0	1.0	9.0
2.0	2.0	8.0
3.0	3.0	7.0
4.0	4.0	6.0
5.0	5.0	5.0
6.0	6.0	4.0
7.0	7.0	3.0
8.0	8.0	2.0
9.0	9.0	1.0
10.0	10.0	0.0

Working with 1 tube at time, add to each tube 10 ml NH_3-cyanide-citrate soln, **24.039(p)**, followed by 30 ml std dithizone soln (30 mg *purified* dithizone dissolved in 1 L $CHCl_3$ and stored in dispensing app. to prevent evapn). Shake vigorously 1 min. and let sep. The pH of aq. phase should be ca 9.4 regardless of whether HCl or HNO_3 is used in rinsing. Stopper each std tube securely with new cork stopper. It is unnecessary to make up entire series of stds if only portion of the range, for example 5.0–10.0 ppm, is of quant. interest.

(**b**) *Comparison.*—Transfer 10 ml portions of clear filtrate from **24.052** to each of 3 Nessler tubes. First add 10 ml NH_3-cyanide-citrate soln, **24.039(p)**, to each tube; to one tube add 30 ml std dithizone soln, (**a**), and to other 2 tubes 30 ml clear $CHCl_3$. Shake tubes vigorously 1 min. and let sep. With tube of clear $CHCl_3$ backing sample tube (contg the dithizone) and 1 sample tube contg $CHCl_3$ backing each of 2 std tubes, compare color in lower layer of sample with that of stds, looking thru tubes at right angles to their lengths toward strong diffused light. (Comparator box similar to boxes used in colorimetric pH measurements but of larger size will be found convenient. When working with apple strip solns, slight turbidity is produced in sample tube, which slightly changes color observed. To compensate for this effect, same turbidity is introduced in field of view of std tubes made up exactly as sample, except that $CHCl_3$ is substituted for the dithizone soln.)

If range is exceeded, i.e., if color produced by sample is redder than the 10 ppm std, repeat with smaller aliquot of filtrate, dilg to 10 ml with "blank" soln. If, for example, 5 ml aliquot is taken, indicated reading must be doubled. After match is obtained, calc. result to basis of 10 ml aliquot and 1400 g sample.

24.054 DETERMINATION WITH PHOTOMETER

(This method is suitable for photometric measurement of the "mixed color," **24.045(b)**. Changes in **24.053** are introduced here to prevent formation of colors too dense for measurements. Use 5 ml instead of 10 ml aliquots of acidified wash soln, **24.052.**)

(**a**) *Standards.*—Measure following proportions of "std" and "blank" solns, **24.053**, into separators:

Pb ppm	"standard" ml	"blank" ml
0.0	0.0	10.0
2.0	1.0	9.0
4.0	2.0	8.0
6.0	3.0	7.0
8.0	4.0	6.0
10.0	5.0	5.0

Add 10 ml NH_3-cyanide-citrate soln, **24.039(p)**, and working with 1 separator at time, immediately develop color by shaking 1 min. with 50 ml pure dithizone soln of 10 mg/L strength. Let stand few min. to cool, filter $CHCl_3$ layers thru specially washed papers, **24.045(b)**, and fill cell of appropriate length (10 mm is convenient). Det. absorbances and plot against ppm Pb to obtain std curve.

(**b**) *Comparison.*—Place appropriate size aliquot of acidified strip soln in separator and dil. to 10 ml with "blank" soln. Add 10 ml NH_3 reagent, **24.039(p)**, and ext. with 25 ml of the std dithizone soln. Let stand few min. to cool, filter, and read as above. Det. quantity of Pb from std curve prepd as in (**a**) and calc. to basis of 5 ml aliquot and 1400 g sample.

24.055 ELECTROLYTIC DETERMINATION OF LEAD IN APPLE FILTRATE

Transfer 200 ml of the acid filtrate, **24.052**, to separator, add equiv. of 5 g citric acid, **24.039(d)**, make ammoniacal, add 5 ml of the 10% KCN soln, ext. with dithizone as in **24.042(a)**, and finally det. Pb electrolytically as in **24.044(b)** and (**c**).

24.056 MANGANESE—FIRST ACTION
—*See* **6.014** or **20.025**

MERCURY (*22*)—OFFICIAL

24.057 PRINCIPLES

Method consists of wet digestion of sample with HNO_3 and H_2SO_4 under reflux in special app., isolation of Hg by dithizone extn, removal of Cu, and estimation of Hg by photometric measurement of Hg dithizonate.

24.058 PRECAUTIONS

Critical step is digestion of sample, which must be almost complete, otherwise residual org. matter may combine with Hg and prevent or hinder extn with dithizone. Oxidizing material in digest must also have been destroyed or dithizone reagent is decomposed and Hg is not quantitatively extd. Because of volatility of Hg compounds, careful heating of digest during sample prepn is required. Acidity of final sample soln (after partial neutralization with NH_4OH) prior

to extn should be ca 1N and not $>1.2N$. Do not use silicone grease in stopcocks.

24.059 APPARATUS

Special digestion apparatus.—See Fig. 46. App. is made from Pyrex with ₮ joints thruout. Unit

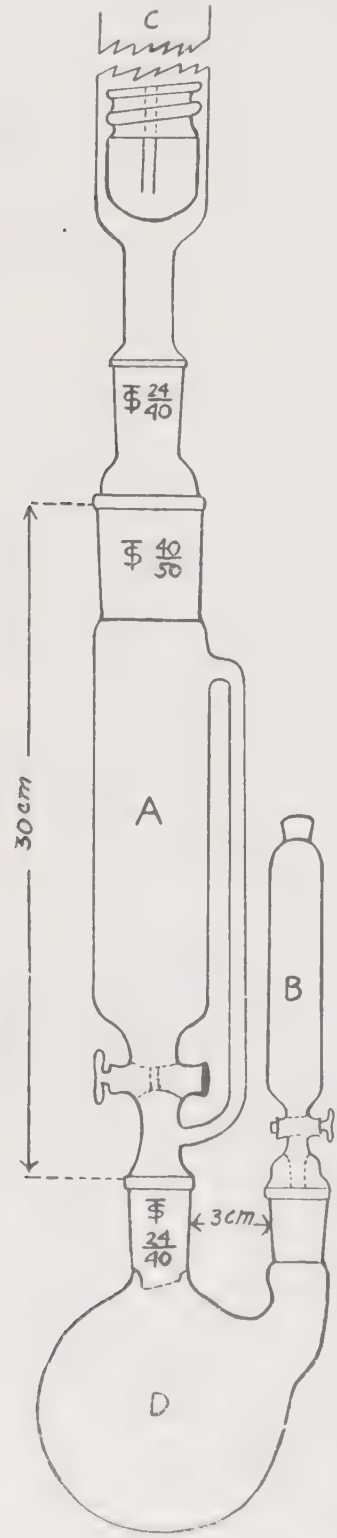

FIG. 46.—SPECIAL DIGESTION APPARATUS

A is modified Soxhlet extractor, 5 cm o. d., 200 ml capacity to overflow, without inner siphon tube but equipped with stopcock on tube leading to digestion flask, D. With stopcock open, app. is in reflux position; when closed, unit serves as trap for condensed H_2O and acids. Top of A is attached to Friedrichs condenser, 35 cm long. Bottom of A is attached thru center neck of 2 neck ₮ 24/40 round-bottom 500 ml flask, D. Necks are 3 cm apart to provide clearance. Second neck is used for attaching 75 ml dropping funnel, B.

NOTE: As Hg compounds tend to adsorb on glassware, app. and particularly separators should be rinsed with dil. HNO_3 and then with H_2O.

24.060 REAGENTS

(a) *Mercury std soln.*—Prep. stock soln contg 1 mg Hg/ml from dry, recrystd $HgCl_2$ (67.7 mg/50 ml). Prep. dil. std solns (2 mmg Hg/ml is convenient) from this stock soln and store in Pyrex bottles. Add HCl in proportion of 8 ml/L to all stds before dilg to final vol.

(b) *Chloroform.—See* **24.012(b)**.

(c) *Dithizone soln.—See* **24.039(e)**. Reagent as now distributed needs no purification for this method. Prep. stock soln in redistd $CHCl_3$ (100 mg/L is convenient) and store in refrigerator. Prep. dilns as needed.

(d) *Sodium thiosulfate soln.*—1.5% w/v. Prep. daily.

(e) *Sodium hypochlorite soln.*—Preferably 5% available Cl reagent. As distributed, reagent varies in available Cl content. Det. strength by analysis. Store in refrigerator when not in use and det. titer monthly. (Certain prepns of hypochlorite intended for household use contain traces of Hg. If these prepns are used, det. blank. Reagent with >0.1 mmg Hg/ml should not be used.)

(f) *Dilute acetic acid.*—30% by vol.

(g) *Hydroxylamine hydrochloride soln.*—20% w/v. Ext. with dil. dithizone until $CHCl_3$ layer remains green, remove excess dithizone with $CHCl_3$, and filter.

24.061 SAMPLE PREPARATION

(Conduct acid digestion in hood)

In all detns use wt sample equiv. to not >10 g dry wt.

(a) *Fresh fruits or vegetables and beverages.*—Place weighed sample in digestion flask with 6 glass beads, connect assembly, and add, thru dropping funnel, 20 ml HNO_3. Pass rapid stream of H_2O thru condenser, adjust stopcock of Soxhlet unit to reflux position, and apply small flame to flask. Use asbestos board with 1–2″ diam. hole between flask and flame. (Original reaction must not proceed violently or evolved NO_2 will carry vapors of digest mechanically thru condenser and

cause loss of Hg.) After initial reaction is complete, apply heat so that digest just refluxes. If mixt. darkens, add HNO_3 dropwise thru funnel as needed. Continue refluxing 0.5 hr, or until digest does not change consistency, and cool.

Add slowly 20 ml cold HNO_3-H_2SO_4 mixt. (1+1). (Use 10 ml acid mixt. for 5 g or less (dry wt) of sample.) Heat with small flame, subsequently adding HNO_3 dropwise as needed to dispel darkening of digest. Continue heating until fibrous material (fruit skin, cellulose, etc.) is apparently digested. Turn stopcock of Soxhlet unit to trap H_2O and acids, and continue heating. Let digest become dark brown (not black) before adding further increments of HNO_3. (Fats and waxes cannot be totally digested by the hot acids under reflux. No attempt should therefore be made to effect complete digestion in this step.) When all but fat and wax is in soln, let digest cool, and drain H_2O and acids cautiously into main digest. Cool, and pour two 25 ml portions H_2O thru condenser and intermediate unit. Remove reaction flask, chill under cold H_2O or by surrounding with ice to solidify fats and waxes, and filter off insol. matter on small pledget of glass wool. Rinse reaction flask and filter pad successively with two 10 ml portions H_2O. Remove Soxhlet unit, and wash it and flask with hot H_2O to remove insol. material. Pour hot H_2O thru condenser to remove volatile fats and oils. Discard all washings.

Connect flask contg filtered sample soln to assembled app., heat, and collect H_2O and acids in trap. Complete digestion, using small addns of HNO_3 as needed. In final stage of digestion, adjust flame until digest reaches incipient boiling (soln simmers) and acid vapors do not rise beyond lower half of condenser. Continue heating 15 min. after last addn of HNO_3. Digest should now be colorless or pale yellow. Let digest cool, drain trapped liquids carefully into reaction flask, and add two 50 ml portions H_2O thru condenser. Reflux soln until all NO_2 is expelled from app. Add 5 ml *40% w/v urea soln* and reflux 15 min. (Digest should be colorless or pale yellow.)

(**b**) *Dried fruit, cereal, seeds, and grains.*—Dil. sample with 50 ml H_2O before adding HNO_3, and proceed with sample prepn as in (**a**).

(**c**) *Meats, fish, and biological material.*—Because of high fat and protein content of these materials, initial digestion must be conducted carefully to avoid foaming of digest into condenser. Add 20 ml HNO_3 to sample, swirl flask, and let stand 0.5 hr in digestion assembly before heating. Add 25 ml H_2O and heat cautiously with small rotating flame until initial vigorous reaction is over and foaming ceases. Proceed as in (**a**).

Titr. 1 ml sample soln thus prepd with std alkali. Add calcd amount of concd NH_4OH to reduce acidity to 1.0N; swirl flask during addn of the NH_4OH to avoid local excess. (Soln should never be ammoniacal to avoid formation of Hg complexes.)

24.062 ISOLATION OF MERCURY

Following table is useful in prepg std curve and for establishing approx. Hg range in sample soln when 10 mm cells are used:

HG RANGE *mmg (0.001 mg)*	DITHIZONE CONCN *mg/L*	VOLUME DITHIZONE *ml*
0–10	6	5
0–50	10	25
0–100	10	40

Transfer sample soln to 500 ml separator. Add 10 ml 4 mg/L dithizone and shake vigorously 1 min. (If green color of dithizone is apparent in $CHCl_3$ layer, indicating excess of dithizone, amount of Hg is within 0–5 mmg.) Let layers sep., and drain $CHCl_3$ layer quickly to second separator contg 25 ml 0.1N HCl and 5 ml of the $NH_2OH.HCl$ soln. (Small amount of oxidizing material may still be present. On long contact with dithizone soln, oxidizing substances may destroy dithizone reagent and prevent extn of Hg.)

Repeat extn of sample soln with two 5 ml portions dithizone soln, transferring $CHCl_3$ layer successively to second separator. If first extn indicates Hg in excess of 5 mmg, add stronger concns of dithizone, as indicated by table, until, after 1 min. vigorous shaking, $CHCl_3$ layer contains dithizone in marked excess. Drain $CHCl_3$ layer into second separator contg the 0.1N HCl and again ext. sample soln with two 10 ml portions 4 mg/L dithizone soln, draining each successive ext. into second separator.

Shake contents of second separator vigorously 1 min., and drain $CHCl_3$ layer into third separator contg 50 ml 0.1N HCl. (Shaking dithizone ext. with dil. acid in second separator removes entrained org. matter which may be present. With biological materials or those of high protein content, aq. layer is usually light yellow because of nitrated org. compounds. Small amounts are carried into third separator where they are destroyed by Cl.) Ext soln in second separator with 1–2 ml $CHCl_3$ and transfer org. layer to third separator.

To contents of third separator add 2 ml of the $Na_2S_2O_3$ soln, shake vigorously 1 min., let layers sep., drain off $CHCl_3$ as completely as possible, and discard. (Cu if present is removed as dithizonate.) Ext. again with with 1–2 ml $CHCl_3$, drain carefully, and discard. Add 3.5 ml of the NaOCl reagent (or enough soln of different titer to furnish 175 mg available Cl) to decompose Hg thiosulfate complex and to oxidize excess thiosulfate, and shake vigorously 1 min. Add 5 ml of the NH_2OH .HCl reagent from pipet, taking care to wet both stopper and neck of separator. Shake vigorously

1 min. Hold mouth of the separator in front of air vent and blow out any remaining gaseous Cl. Stopper separator and shake vigorously 1 min. (It is imperative that all hypochlorite be reduced. Traces of the reagent remaining would oxidize dithizone, subsequently added, to yellow oxidized form which would be measured in photometer as Hg.) Ext. soln with 2–3 ml $CHCl_3$, drain off org. layer carefully, and discard. Final aq. soln should now be colorless.

24.063 DETERMINATION

To contents of third separator add 3 ml of the dil. HOAc and appropriate vol. and concn of dithizone soln as indicated by table, **24.062,** and proceed with colorimetric detn of Hg as in **24.064,** converting absorbance, measured at 490 mμ to mmg Hg from working curve.

24.064 PREPARATION OF STANDARD CURVE

Prep. working curve of required range, starting with blank and extending to final std of range, with 4 intermediate increasing increments. Add appropriate amounts of Hg to 50 ml 0.1*N* HCl in separator. Add 5 ml of the $NH_2OH.HCl$ reagent and 5 ml $CHCl_3$, and shake vigorously 1 min. Let layers sep., drain off $CHCl_3$, and discard, being careful to remove as completely as possible all droplets of $CHCl_3$. Add 3 ml of the dil. HOAc and appropriate vol. dithizone soln, shake vigorously 1 min., and let layers sep. (HOAc aids in stabilizing mercuric dithizonate.) Insert cotton pledget into stem of separator and collect dithizone ext. (discarding first ml) in test tube for transfer to appropriate cell. Make photometer readings at 490 mμ. (Since both dil. dithizone and mercuric dithizonate are somewhat unstable, det. photometric readings immediately.) Plot absorbance against mmg Hg.

SELENIUM (*23*)—OFFICIAL

24.065 PRINCIPLES

Method consists of wet digestion with HNO_3 and H_2SO_4 in presence of HgO fixative, sepn of Se by distn as volatile bromide, reduction of the bromide to elementary Se with SO_2, isolation, and estimation as H_2SeO_3 by titrn with std $Na_2S_2O_3$ and I.

24.066 REAGENTS

(a) *Sulfuric-nitric acid soln.*—To 50 ml H_2SO_4 add 100 ml HNO_3. Cool mixt. before using.

(b) *Mercuric oxide fixative.*—Dissolve HgO in HNO_3 in proportion of 5 g/100 ml of the acid.

(c) *Concentrated hydrobromic acid-bromine soln.*—Mix 10 ml liquid Br with 990 ml HBr. (Reagent-grade HBr is available commercially in 2 concns: constant boiling mixt. of 48% (8.1*N*) and the 40% concn (7*N*). Either may be used, as detn allows for varying quantity of reagent taken according to HBr concn.)

(d) *Dilute hydrobromic acid-bromine soln.*—To 5 ml HBr add 10 ml satd Br-H_2O and dil. to 100 ml with H_2O.

(e) *Sulfur dioxide.*—Gas supplied in commercial cylinders is free of Se.

(f) *Hydroxylamine hydrochloride soln.*—10%, w/v.

(g) *Phenol soln.*—5%, w/v.

(h) *Sodium thiosulfate std soln.*—Prep. from accurately stdzd 0.1*N* reagent with recently boiled H_2O. Before adjusting to final vol., add 5 ml *iso*-amyl alcohol/L and shake vigorously. 1 ml 0.001*N* $Na_2S_2O_3$ is theoretically equiv. to 19.8 mmg Se. (For estimation of Se in quantities >50–75 mmg, proportionately higher concns of $Na_2S_2O_3$ are required.)

(i) *Iodine std soln.*—Prep. from 0.1*N* reagent. Before final diln add KI in proportion of 20 g/L. Dil. to same normality as the $Na_2S_2O_3$ soln.

(j) *Selenium std soln.*—Dissolve 250 mg Se in concd HBr-Br soln (1 ml liquid Br + 25 ml concd HBr, both of which have been distd. After soln is complete, *almost* neutralize excess Br with the SO_2, while shaking vigorously. Complete neutralization by adding the phenol soln dropwise in slight excess. Dil. to 250 ml with H_2O. (SO_2 must not be present in excess because it would then reduce H_2SeO_3 to Se.) If too much SO_2 has been used, add Br-H_2O until color of selenite soln is slightly but definitely yellow and then complete neutralization with the phenol soln.

(If Se reagent is not pure, purify it as follows: Dissolve ca 1 g Se in excess of the concd HBr-Br soln, ppt with SO_2, warm on steam bath 30 min., cool, filter, first wash free of acids with H_2O and then wash with small portions of alcohol, dry 1 hr at 100°, and prep. the 1 mg/ml std as directed previously. This precaution is necessary since Se soln serves as ultimate std in detn.)

Make appropriate dilns of concd std soln by adding H_2O, and do not let acidity, detd by titrn, fall to <0.05*N*, because neutral or very slightly acid solns of dil. H_2SeO_3 tend to oxidize and lose titer. Diln of 20 mmg Se/ml is convenient for micro detns, for then it is almost chemically equiv. to accurately prepd 0.001*N* $Na_2S_2O_3$ (1 ml 0.001*N* $Na_2S_2O_3$ = 19.8 mmg Se.)

24.067 APPARATUS

All-glass distn app. consisting of 250 ml round-bottom flask, still head, thermometer registering to 135°, and condenser with dipping end.

24.068 DETERMINATION

Place 5–10 g (dry wt) sample in 600–800 ml Pyrex beaker and add 10 ml of the HgO fixative

followed by 150 ml of the H_2SO_4-HNO_3 soln. Mix thoroly at once and place on steam bath 30 min., stirring intermittently. If product is high in Se, use 1 g representative material. To dry leafy products which oxidize violently, add 25 ml H_2O before adding fixative. Heat over burner (not full flame) until digestion mass lightens and then turns brown. Remove flame, cool, and after adding 10 ml HNO_3, again heat until first brown appears. Repeat this operation at least twice and then heat until liquid turns distinct brown (not black) or until SO_3 fumes appear. (It is imperative to expel excess HNO_3 and to oxidize org. matter sufficiently so that Br reagent subsequently added is not reduced, but prolonged fuming to SO_3 is to be avoided.) As such products as molasses and honey, principally sugars, react vigorously with HNO_3, remove such samples from steam bath until reaction subsides and then proceed in usual manner.

Cool digest and transfer with two 25 ml portions H_2O to distg flask. (If digestion is performed in the 250 ml distg flask, it is necessary even then to add 50 ml H_2O so that HBr will distill subsequently as liquid and not as vapor.) Rinse beaker carefully with 25 ml of the HBr-Br soln and add to cooled digest and washings. (If constant-boiling grade of HBr has not been used, equiv. vol. of less concd reagent must be added, e.g., 30 ml 40% concn, and distillate must have acidity of ca 2.5N.) After swirling flask, distill, until temp. of distn reaches 130°, into 125 ml erlenmeyer, marked at 50, 75, and 100 ml, contg 5 ml HBr and surrounded by cold H_2O. During distn lift tip of condenser out of liquid in flask after all Br and ca 15 ml of the acid have distd. (Free Br should distill in beginning, indicating excess of reagent. If this is not the case, stop distn, cool, and add addnl 10 ml of the HBr-Br soln. This contingency arises only with insufficient digestion of sample.) Rinse condenser tip carefully with 2 portions of not >2 ml each of H_2O. Between analyses rinse condenser tube free of fatty and waxy material with hot H_2O but *do not* add rinsings to distillate. For next 3 steps it is assumed that distillate contains no fats, waxes, or other insol. matter.

(1) If vol. distillate and rinsings is 75 ml or less, pass in SO_2 in excess (ca 30 sec. after complete decolorization of Br), add 1 ml of the $NH_2OH.HCl$ soln, and place mixt. on active steam bath 30 min. Cap flasks with watch glasses during various heat treatments.

(2) If vol. distillate is 75–100 ml, reduce with SO_2 and the $NH_2OH.HCl$ soln as in (1), add several glass beads, bring just to incipient boiling, and complete reduction at once with the 30-min. steam-bath treatment.

(3) If vol. distillate is >100 ml, transfer to 200 ml erlenmeyer and complete transfer with 4 successive 2 ml H_2O rinsings delivered from pipet. Add 10 ml HBr, reduce with SO_2 and the NH_2OH .HCl, add several glass beads, bring to incipient boiling, and place flask at once on steam bath and heat 30 min. (When vol. soln is >100 ml, recovery of Se may be slightly low.)

If distillate contains fats, waxes, or other insol. material, filter off with suction on asbestos and rinse receiver flask carefully with four 2 ml portions H_2O from pipet. Use successive washings in turn to rinse asbestos filter. Transfer combined filtrates to 125 ml erlenmeyer (200 ml flask if vol. filtrate is >100 ml), and complete transfer with four 2 ml portions H_2O, delivered from pipet. According to final vol. soln, whether <75 ml, between 75 and 100 ml, or >100 ml, proceed with addn of reagents and heat treatment exactly as above. (Se is reduced rapidly from acid soln 2.5N or greater; steps (2) and (3) are necessary because acidity is <2.5N in these instances.)

For assay of products of high Se content (>1.0 mg in sample analyzed) use 50 ml of the HBr-Br soln (or equiv. vol. of less concd grade) for initial distn. To such samples add 75 ml H_2O during rinsing instead of usual 50 ml. After usual distn, disconnect app., rinse condenser tube with 5 ml H_2O, and add rinsing directly to distillate. Heat residue in distn flask to incipient fumes of SO_3, cool, add 5 ml $HClO_4$, and heat to fuming. Repeat $HClO_4$ oxidation. (This treatment is necessary for substances like vetches and seedlings, which contain particularly refractory Se compounds.) Cool digest, add two 25 ml portions H_2O and then 25 ml of the HBr-Br soln, and distill to 130° in usual manner. Combine all distillates, and if fats, waxes, or other insol. matter is present, filter, and wash as previously directed. In either case, adjust to 250 ml in vol. flask with H_2O, pipet 75 ml into 125 ml erlenmeyer, reduce Se with excess SO_2 and $NH_2OH.HCl$, and complete reduction by heating on steam bath 30 min.

Place flasks in cold H_2O (ca 20°) for 30 min. and then, with suction, collect the Se on asbestos pad contained in filtration vessel, Fig. 47-A. Rinse pptn flask and pad with 5 successive 1 ml portions H_2O from pipet, and then hold mouth of flask before air vent to remove last traces of SO_2.

Insert filtration vessel into titrg tube and dissolve the Se with 1 ml of the dil. HBr-Br soln, first adding reagent from pipet to flask and then transferring carefully to pad. When Se has dissolved, apply gentle suction and repeat operation with addnl 1 ml of the dil. HBr-Br soln. Finally rinse flask and pad with 3 successive 1 ml portions H_2O, collecting filtrate before each addn; 2 ml of the dil. HBr-Br soln is enough for Se up to 500 mmg. When more is present, use proportionately more reagent and rinse H_2O.

Agitate filtrate with pipet stirrer and dispel excess Br with 3 drops of the phenol soln. Using stirrer as pipet, rinse walls of vessel several times with the soln to neutralize every trace of Br. Immerse titrg tube up to $\frac{2}{3}$ its length in hot H_2O 5 min., stirring intermittently. (Heating is required to complete reaction between Br and phenol.) Then place vessel in cold H_2O at least 5 min. (Norris-Fay titrn (*24*) works best when soln is $<25°$.)

Using original ppt of Se as guide, add at least 50% excess of appropriate concn of std $Na_2S_2O_3$ soln and 3 drops starch indicator, **24.039(h)**. After stirring, add std I until permanent blue appears. If <1 ml I soln is required, add enough $Na_2S_2O_3$ so that at least 1 ml I is required. Then titr. to colorless end point with $Na_2S_2O_3$ soln, adding reagent in 0.01 ml portions as end point is approached.

24.069 STANDARDIZATION OF IODINE

(Cross titration)

To 2 ml HBr (5+95) contained in titrg tube add 3 ml H_2O and 3 ml std I. (The HBr must have been previously distd.) Titr. with std $Na_2S_2O_3$ soln and toward end add 3 drops starch indicator, **24.039(h)**. Complete titrn as in **24.068**, and obtain $Na_2S_2O_3$ equiv. of the I.

24.070 STANDARDIZATION OF THIOSULFATE

Add 2 ml of the dil. HBr to appropriate vol. of std Se soln, and after adding 50% excess $Na_2S_2O_3$ soln continue titrn exactly as in **24.069.** Obtain Se equiv. of the $Na_2S_2O_3$ soln. (As dil. solns of both $Na_2S_2O_3$ and I always slowly deteriorate, they must be stdzd frequently.)

24.071 SAMPLE CALCULATION

Net $Na_2S_2O_3$ sample titer in ml$\times$Se equiv. = quantity of Se in sample.

TIN—FIRST ACTION

24.072 PREPARATION OF SAMPLE

Digest 50–100 g sample as in **24.003.**

Gravimetric Method (25)

24.073 REAGENTS

(a) *Wash soln.*—Mix 100 ml satd NH_4OAc soln with 50 ml HOAc and 850 ml H_2O.

(b) *Ammonium polysulfide soln.*—Pass H_2S gas into 200 ml NH_4OH in bottle immersed in running H_2O or in ice-H_2O until gas is no longer absorbed; add 200 ml NH_4OH and dil. with H_2O to 1 L. Digest this soln with 25 g flowers of S several hrs and filter.

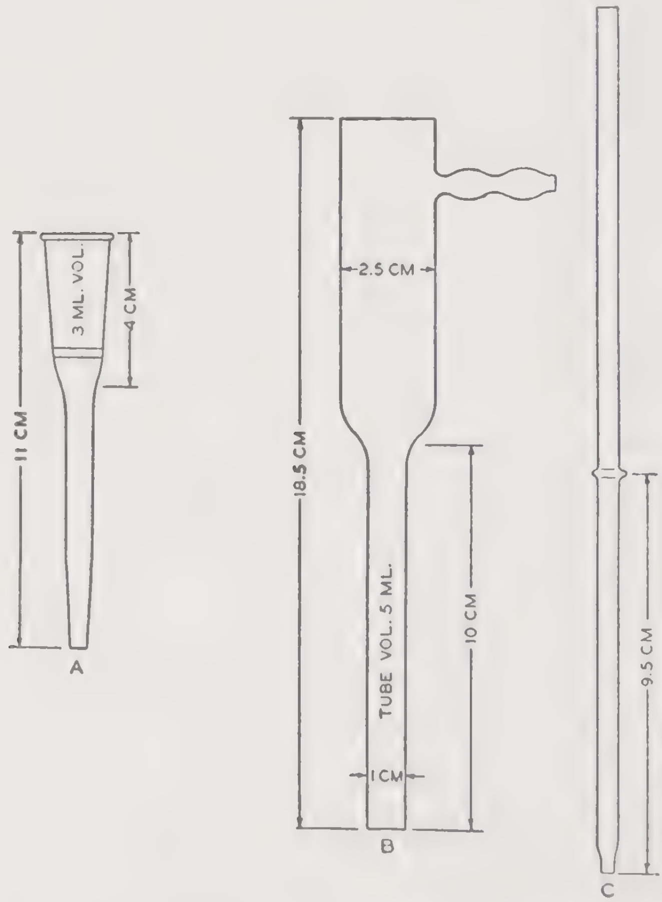

FIG. 47.—FILTRATION AND TITRATION TUBES AND PIPET

24.074 DETERMINATION

Add 200 ml H_2O to digested sample and transfer to 600 ml beaker. Rinse Kjeldahl flask with 3 portions of boiling H_2O, making total ca 400 ml. Cool, and add NH_4OH until just alk.; then add 5 ml HCl or 5 ml H_2SO_4 (1+3) for each 100 ml soln. Place beaker, covered, on hot plate; heat to ca 95° and pass in slow stream of H_2S 1 hr. Digest 1 hr at 95° and let stand 30 min. longer.

Filter, and wash ppt of SnS alternately with 3 portions each of the wash soln and hot H_2O. Transfer filter and ppt to 50 ml beaker, add 10–20 ml of the $(NH_4)_2S_x$ soln, heat to boiling, and filter. Treat contents of beaker with 2 addnl portions of hot $(NH_4)_2S_x$ soln and wash filter with hot H_2O. Acidify combined filtrate and washings with HOAc (1+9), digest on hot plate 1 hr, let stand overnight, and filter thru double 11 cm paper. Wash alternately with 2 portions each of the wash soln and hot H_2O and dry thoroly in weighed porcelain crucible. Ignite over Bunsen flame, very gently at first to burn off paper and to convert sulfide to oxide; then partly cover crucible and heat strongly over large Bunsen or Meker burner. (SnS must be roasted gently to SnO_2, which then may be heated to high temp. without loss by volatilization.) Weigh as SnO_2 and calc. to metallic Sn, using factor 0.7877.

Volumetric Method (26)

24.075 REAGENTS

(**a**) *Air-free wash soln.*—Dissolve 20 g $NaHCO_3$ in 2 L boiled H_2O and add 40 ml HCl. This soln should be freshly prepd.

(**b**) *Iodine std soln.*—0.01*N*. Stdze soln frequently against (**c**), adding asbestos mat and proceeding as in **24.076,** omitting pptn with H_2S and boiling with HCl and $KClO_3$. Quantity of Sn in soln used for stdzn should equal ca that contained in sample under examination.

(**c**) *Tin std soln.*—Dissolve 1 g Sn in ca 500 ml HCl and dil. to 1 L with H_2O. (1 ml = 1 mg Sn.)

(**d**) *Sheet aluminum.*—About 30 gauge, free from Sn.

24.076 DETERMINATION

Proceed as in **24.074** thru "Digest 1 hr at 95° and let stand 30 min. longer."

Filter thru asbestos in Caldwell crucible, using suction. Wash ppt of SnS few times with H_2O and transfer detachable bottom and asbestos pad to 300 ml erlenmeyer. Remove all traces of ppt from inside of crucible, using jet of hot H_2O and policeman, and using min. quantity of H_2O for washing.

Add to flask 100 ml HCl and 0.5 g $KClO_3$. Boil ca 15 min., making ca 4 more addns of smaller quantities of the $KClO_3$ as Cl is boiled out of soln. Wash particles of $KClO_3$ down from neck of flask with H_2O and finally boil to remove Cl. Add ca 1 g of the sheet Al to dispel last traces of Cl.

Fit 2 hole rubber stopper to flask. Thru 1 hole pass bulbed glass tube that reaches nearly to surface of liquid. Attach this tube to large CO_2 generator thru scrubber contg H_2O. The CO_2 passes out of flask thru short, bulbed tube inserted in second hole of stopper and ending slightly below it. With rubber tube connect this second glass tube to another glass tube, ca 10″ long, immersed in cylinder of H_2O to depth of ca 8″. (This connection acts as seal to restrain any strong flow of gas when not desired and to permit pressure in flask.)

Raise delivery tube nearly out of H_2O seal, thus allowing rapid flow of CO_2 for few min. to dispel air from system. Then lower delivery tube into H_2O seal, slightly raise stopper, and quickly drop into flask 1–2 g of the sheet Al, folded into narrow bent strip to prevent breaking flask. After Al dissolves completely raise tube in H_2O seal, letting CO_2 pass thru rapidly; place flask on hot plate and boil few min. Remove flask from heat and cool with tap or ice-H_2O, continuing flow of CO_2. Lower delivery tube into cylinder, disconnect flask, and, with glass plug, close rubber tube thru which CO_2 enters flask. Wash glass tubes, rubber stopper, and sides of flask with the air-free wash soln; add starch indicator, **24.039**(**h**), and titr. immediately with the 0.01*N* I.

If desired make titrn by slightly raising rubber stopper after cooling and adding excess of the 0.01*N* I. Then disconnect flask; wash tubes, rubber stopper, and sides of flask with the air-free wash soln; and titr. excess I with 0.01*N* $Na_2S_2O_3$.

ZINC—FIRST ACTION

Gravimetric Method

(Min. of 2 mg Zn)

24.077 REAGENTS

(**a**) *Sodium or ammonium acetate soln.*—Dissolve 50 g of the salt in H_2O and dil. to 100 ml.

(**b**) *Ferric chloride soln.*—Dissolve 10 g $FeCl_3.6H_2O$ in 100 ml H_2O.

24.078 DETERMINATION

Boil filtrate contg Zn obtained after filtering off CuS, **24.018,** to expel H_2S and reduce vol. to 250–300 ml, add drop Me orange and 5 g NH_4Cl, and make alk. with NH_4OH. Add HCl (1+9) dropwise to faintly acid reaction; then add 10–15 ml of the NaOAc or NH_4OAc soln and pass in H_2S until pptn is complete. Let ppt settle, filter (clear filtrate is necessary), and wash ppt twice with satd H_2S soln. Dissolve ppt on filter with little HCl (1+3), wash filter with H_2O, boil combined filtrate and washings to expel H_2S, cool, and add

distinct excess of Br-H_2O. Add 5 g NH_4Cl and then NH_4OH until color of free Br disappears. Add HCl (1+3) dropwise until Br color just reappears; then add 10–15 ml of the NaOAc or NH_4OAc soln and 0.5 ml of the $FeCl_3$ soln, or enough to ppt all phosphates. Boil until all Fe is pptd. Filter while hot and wash ppt with H_2O contg little NaOAc.

Pass H_2S into combined filtrate and washings until all ZnS, which should be pure white, is pptd. Filter thru weighed gooch, previously heated to constant wt, and wash with H_2S soln contg little NH_4NO_3. Dry crucible and contents in oven, ignite at bright red heat (900°), cool, and weigh as ZnO. Calc. wt to metallic Zn, using factor 0.8034.

Colorimetric Method (27)

24.079 PRINCIPLES

This method involves wet oxidation of sample; elimination of Pb, Cu, Cd, Bi, Sb, Sn, Hg, and Ag as sulfides with added Cu as scavenger agent; simultaneous elimination of Co and Ni by extg metal complexes of α-nitroso-β-naphthol and dimethylglyoxime, resp., with $CHCl_3$; extn of the Zn dithizonate with CCl_4; transfer of Zn to dil. HCl; and final extn of Zn dithizonate for color measurement.

24.080 REAGENTS

All H_2O must be redistd from glass. Pyrex glassware should be used exclusively and must be scrupulously cleaned with hot HNO_3. Purify HNO_3 (usually unnecessary) and NH_4OH by distn in Pyrex if appreciably contaminated. Test H_2SO_4 if Zn contamination is suspected.

(a) *Copper sulfate soln.*—Dissolve 8 g $CuSO_4.5H_2O$ in H_2O and dil. to 1 L. (1 ml = 2 mg Cu.)

(b) *Ammonium citrate soln.*—Dissolve 225 g $(NH_4)_2HC_6H_5O_7$ in H_2O, make alk. to phenol red with NH_4OH (pH 7.4, first distinct color change), and add 75 ml in excess. Dil. to 2 L. Ext. this soln immediately before use as follows: Add slight excess of dithizone and ext. with CCl_4 until solvent layer is clear bright green. Remove excess dithizone by repeated extn with $CHCl_3$, and finally ext. once more with CCl_4. (It is essential that excess dithizone be entirely removed, otherwise Zn will be lost during elimination of Co and Ni.)

(c) *Dimethylglyoxime soln.*—Dissolve 2 g reagent in 10 ml NH_4OH and 200–300 ml H_2O, filter, and dil. to 1 L.

(d) *α-Nitroso-β-naphthol soln.*—Dissolve 0.25 g in $CHCl_3$ and dil. to 500 ml.

(e) *Chloroform.*—Redistd.

(f) *Diphenylthiocarbazone (dithizone) soln.*—Dissolve 0.050 g dithizone in 2 ml NH_4OH and 100 ml H_2O, and ext. repeatedly with CCl_4 until solvent layer is clear bright green. Discard solvent layer and filter aq. portion thru washed ashless paper. (This soln is best prepd as needed since it is only moderately stable, even when kept in dark and under refrigeration.)

(g) *Carbon tetrachloride.*—Redistd.

(h) *Dilute hydrochloric acid.*—0.04*N*. Dil. required quantity of HCl with H_2O (redistd acid may be used altho usually unnecessary).

(i) *Zinc std soln.*—Dissolve 0.500 g pure granulated Zn in slight excess of dil. HCl and dil. to 1 L. For use, dil. 10 ml of this stock soln to 1 L with 0.04*N* HCl. (1 ml = 5 mmg Zn.)

24.081 PREPARATION OF SAMPLE

Weigh, into suitable size erlenmeyer, representative sample not >25 g material, estimated to contain 25–100 mmg Zn. If sample is liquid, evap. to small vol. Add HNO_3 and heat cautiously until first vigorous reaction subsides somewhat; then add 2–5 ml H_2SO_4. Continue heating, adding more HNO_3 in small portions as needed to prevent charring, until fumes of SO_3 evolve and soln remains clear and almost colorless. Add 0.5 ml $HClO_4$ and continue heating until it is almost completely removed. Cool, and dil. to ca 40 ml. (Wet digestion and subsequent sulfide sepn may also be advantageously carried out in small Kjeldahl flask.)

24.082 SEPARATION OF SULFIDE GROUP

To the H_2SO_4 soln add 2 drops Me red and 1 ml of the $CuSO_4$ soln, and neutralize with NH_4OH. Add enough HCl to make soln ca 0.15*N* with respect to this acid (ca 0.5 ml excess in 50 ml soln is satisfactory). The pH of soln at this point as measured with glass electrode is 1.9–2.1. Pass stream of H_2S into soln until pptn is complete. Filter thru fine paper (Whatman No. 42 or equiv., previously fitted to funnel and washed with HCl (1+6), then with redistd H_2O). Receive filtrate in 250 ml beaker, and wash flask and filter with 3 or 4 small portions of H_2O. Boil filtrate gently until odor of H_2S can no longer be detected; then add 5 ml satd Br-H_2O and continue boiling until Br-free. Cool, neutralize to phenol red with NH_4OH, and make slightly acid with HCl (excess of 0.2 ml 1+1 HCl). Dil. resultant soln to vol. For optimum conditions of measurement, soln should contain 0.2–1.0 mmg Zn/ml.

24.083 ELIMINATION OF NICKEL AND COBALT

Transfer 20 ml aliquot of prepd soln to 125 ml separator; add 5 ml of the NH_4 citrate soln, 2 ml of the dimethylglyoxime soln, and 10 ml of the α-nitroso-β-naphthol soln; and shake 2 min. Discard solvent layer and ext. with 10 ml $CHCl_3$ to

remove residual α-nitroso-β-naphthol. Discard solvent layer.

24.084 ISOLATION AND ESTIMATION OF ZINC

To aq. phase following removal of Ni and Co, which at this point has pH of 8.0–8.2, add 2.0 ml of the dithizone soln and 10 ml CCl_4, and shake 2 min. Let phases sep. and remove aq. layer as completely as possible, withdrawing liquid with pipet attached to vac. line. Wash down sides of separator with ca 25 ml H_2O and without shaking again draw off aq. layer. Add 25 ml of the 0.04*N* HCl and shake 1 min. to transfer Zn to acid-aq. layer. Drain and discard solvent, being careful to dislodge and remove drop that usually floats on surface. To acid soln add 5.0 ml of the NH_4 citrate soln and 10.0 ml CCl_4 (pH of soln at this point is 8.8–9.0).

Det. quantity of dithizone to be added as follows: To separator contg 4.0 ml of the working Zn std (20 mmg), dild to 25 ml with the 0.04*N* HCl, 5.0 ml of the citrate buffer, and 10.0 ml CCl_4, add the dithizone reagent in 0.1 ml increments, shaking briefly after each addn until faint yellow in aq. phase indicates bare excess of reagent. Multiply vol. dithizone soln required by 1.5 and add this vol. (to nearest 0.05 ml) to all samples. Shake 2 min. Pipet exactly 5.0 ml solvent layer into clean, dry test tube, dil. with 10.0 ml CCl_4, mix, and det. transmittance (or absorbance) at 540 mμ.

24.085 PREPARATION OF STANDARD CURVES

Prep. series of separators contg 0, 5, 10, 15, and 20 mmg Zn dild to 25 ml with the 0.04*N* HCl; add 5.0 ml of the citrate buffer, and proceed as with final extn of Zn, **24.084.**

Plot transmittance on logarithmic scale (or absorbance on linear scale) against concn and draw smooth curve thru points. (Intercept of this curve may vary slightly from day to day, depending on actual concn of dithizone used in final extn, but slope should remain essentially same.)

ORGANIC RESIDUES

ARAMITE (*2-(p-TERT*-BUTYLPHENOXY)-1-METHYLETHYL 2-CHLOROETHYL SULFITE)(*28*)—FIRST ACTION

PRINCIPLES

Aramite is stripped with benzene, the strip soln is concd, and the Aramite is hydrolyzed with KOH-isopropanol to form ethylene oxide. Evolved ethylene oxide is converted to HCHO with KIO_4 and the HCHO is reacted with acetylacetone to form colored compound.

24.086 REAGENTS

(a) *Isopropyl alcohol.*—Redistill and store over anhyd. Na_2SO_4.

(b) *Periodic acid soln.*—Dissolve 0.5 g in H_2O and dil. to 100 ml with H_2O. Dil. 1 vol. with 2 vols. 0.1*N* HCl.

(c) *Sodium arsenite soln.*—Dissolve 2 g in H_2O and dil. to 100 ml with H_2O.

(d) *Acetylacetone reagent.*—Dissolve 25 g NH_4OAc in H_2O, add 3 ml HOAc and 0.2 ml redistd acetylacetone, and dil. to 100 ml with H_2O.

(e) *Aramite std soln.*—Accurately weigh ca 250 mg (ca 0.25 ml) std Aramite (available from Naugatuck Chemical Div., U. S. Rubber Co., Naugatuck, Conn.), dissolve in benzene, and dil. with benzene to 50 ml. Dil. aliquot contg 5 mg to 100 ml with benzene. (1 ml = 50 mmg.) Dil. this soln further to obtain lower concns. Solns as dil. as 10 or 25 mmg/ml remain unchanged 2–3 weeks. Store std solns in refrigerator.

24.087 ISOLATION APPARATUS

Lead air or N at ca 1 lb pressure into surge tank consisting of 2 L bottle fitted with inlet and outlet. Connect outlet to 500 ml gas washing bottle contg H_2SO_4, connected thru capillary bubble counter (constructed from thermometer tubing) filled with light mineral oil, and then to reaction tube (25 × 100 mm over-all length with ₸ 19/22 ground glass joint) with gas inlet tube equipped with inner ₸ 19/22 joint to fit into reaction tube. Attach at least 80 mm 8 mm o.d. thin wall tubing above inlet before attachment to 2–5 mm i.d. capillary delivery tube. (Gas inlet tube, Corning No. 96800, ₸ 19/38, may be used by connecting a ₸ 19/38 inner joint to capillary delivery tube.) Construct scrubber at lower end of delivery tube with 4 expanded rings in 50 mm, which just fits inside 11 mm i.d. test tube, upper portion of which is enlarged to 16 mm i.d. to increase its vol. (*See* Fig. 48).

24.088 PREPARATION OF SAMPLES

(a) *Leafy vegetables.*—Chop into small pieces. Weigh 500 g into 2 qt tumble jar, add 500 ml benzene, and roll or tumble ca 10 min. Pour off solvent, sep. any H_2O, dry benzene with anhyd. Na_2SO_4, and filter.

(**b**) *Small fruits.*—Weigh 500 g into 2 qt tumble jar and strip with 500 ml benzene as above.

(**c**) *Large fruits.*—Weigh 10 whole fruits (1–2 kg) into 2 gallon tumble jar, add 500 ml benzene, and roll or tumble ca 10 min. Pour off benzene, dry, and filter as above.

24.089 DETERMINATION

Conc. aliquot of filtered strip soln (100–400 ml) to 3 ml in reaction tube. (Entire strip soln may be concd in beaker under gentle air current and dild to definite vol. (50 or 100 ml). Aliquots of this concn can then be measured directly into reaction tube for final evapn to 3 ml. The soln must *never* be evapd to dryness.) Place 3 ml premixed reagent, **24.086**(**b**), in scrubber. (Use 3 ml for residues <0.5 ppm or 5 ml for residues >0.5 ppm. Two std curves are required, 1 for 3 ml vol. in scrubber and other for 5 ml.) To sample in reaction tube add 5 ml isopropyl alcohol and 3 pellets KOH. Attach to app. without delay.

Pass current of dry air or N thru gas inlet tube at rate of 4–6 ml/min., and heat reaction tube, maintaining *gentle* refluxing 30 min. Remove scrubber tube and heat in 50° constant temp. bath 15 min. Cool to room temp. Pipet 2 ml into small test tube, add 0.5 ml 2% sodium arsenite, let stand for moment, shake, and let stand until colorless. Add 0.5 ml acetylacetone reagent, mix, and heat in boiling H_2O or live steam 6 min. Remove from heat and let stand at least 10 min. in air. Cool to room temp., transfer to cuvette, and det. absorbance at 412 mμ. Color is stable for at least 2 hr. Det. mmg from std curve, and calc. to ppm.

Prep. std curve by measuring quantities of std soln contg 0–150 mmg into reaction tube with enough benzene to total 3 ml, and carry thru entire detn as for samples. Plot absorbances against mmg Aramite.

BENZENE HEXACHLORIDE (HEXACHLOROCYCLOHEXANE, BHC)(*29*)—OFFICIAL

24.090 PRINCIPLES

BHC is removed from sample by extg with CCl_4. After removal of solvent, BHC is dechlorinated to benzene by action of Zn and HOAc in presence of malonic acid which slowly liberates CO_2, sweeping benzene formed into nitrating mixt. Benzene is converted in constant but not quant. (ca 85%), proportion to *m*-dinitrobenzene. After extn, *m*-dinitrobenzene is treated with butanone-2 and alkali, and absorbance of magenta-colored compound formed is measured at 565 mμ.

24.091 APPARATUS

(**a**) *Glass gooch holders.*—Body ca 25 mm diam. by 75 mm long; stem ca 30 mm long.

(**b**) *All-glass digestion and nitrating appa-*

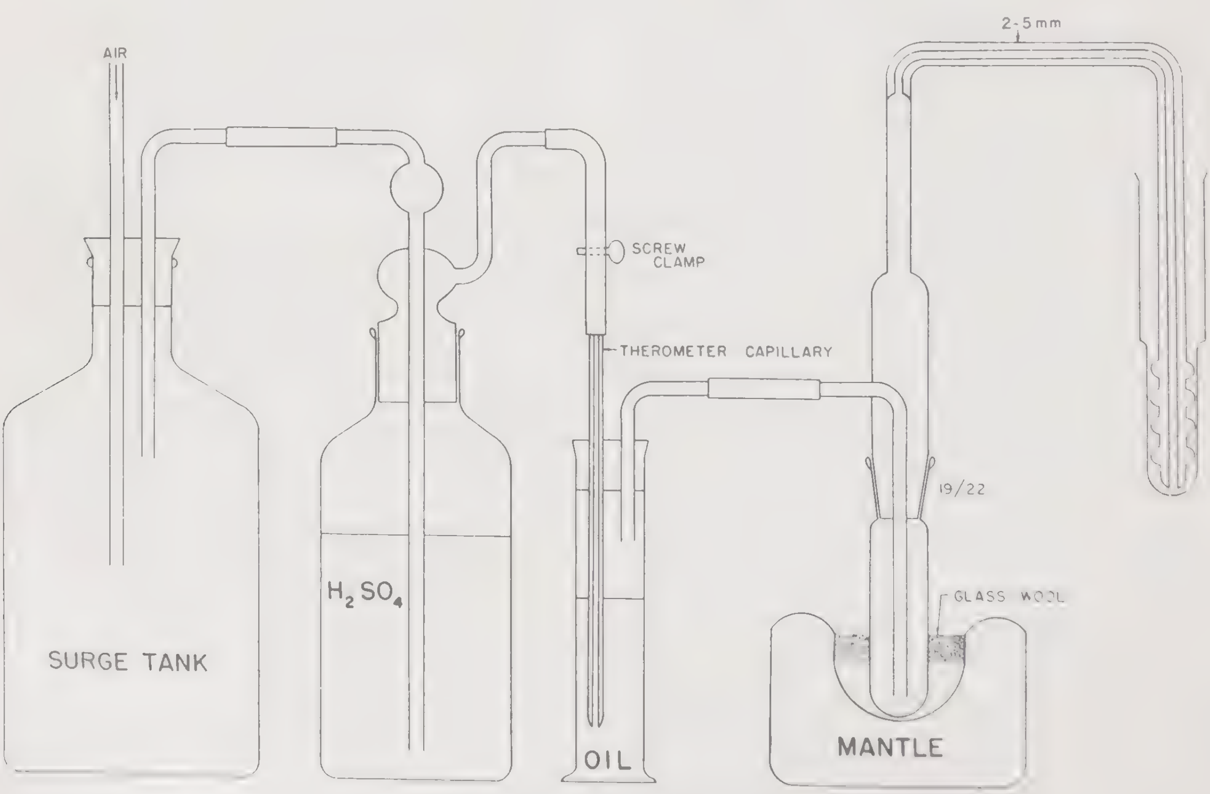

FIG. 48.—APPARATUS FOR DETERMINATION OF ARAMITE

ratus.—*See* Fig. 49. *A*: Outer jacketing tube, 28 mm o.d.×35 cm to ring seal. *B*: Glass tube 16 mm o.d.×26 cm to ring seal. *C*: Glass tube 9 mm o.d. *D*: 19/38 ₸ joint. *E*: Reaction flask with 19/38 ₸ joint. *F*: Nitrating tube, 9 mm o.d., flared at top. *G*: Solid glass beads 3 mm diam., packed to height of ca 15 cm. *H*: Glass wool plug. *I*: Stopcock, 2 mm oblique bore.

Between detns dry app. as follows: Suck out visible H_2O with vac. Remove vac., and pour acetone thru nitrating unit and inner tubes twice. Attach dry, clean reaction flask and apply vac. to spigot at least 15 min.

(c) *Electric heating units.*—Fisher No. 11-502-15 or equiv., screwed into porcelain socket. Elec. hot plate may be used if app. is placed with reaction flask at edge of hot plate and nitrating tube extending away from hot plate.

(d) *Reaction flask.*—50 ml flat-bottom, ₸ 19/38.

24.092 REAGENTS

(a) *Malonic acid.*—Reagent grade.

(b) *Glacial acetic acid.*—If reagent blank exceeds 10 mmg apparent BHC/15 ml, purify by redistg in all-glass app., rejecting first 20% distillate. (This operation diminishes blank by half.) Det. blank on each new lot of reagent.

(c) *Nitrating acid.*—Mix fuming HNO_3 (sp. gr. 1.49–1.50) with equal vol. H_2SO_4 (sp. gr. 1.84).

(d) *Ether.*—If evapn of 100 ml leaves yellow or light brown residue, redistill before using.

(e) *Potassium hydroxide soln.*—40% w/v. Dissolve 470 g KOH (85%) in H_2O and dil. to 1 L.

(f) *Mineral oil.*—Refined, aromatic-free.

(g) *Methyl ethyl ketone (butanone-2).*—Fractionate and collect portion distg at 79–81°.

(h) *Absorbent cotton.*—Purify as follows: Immerse in ether, stir 5 min., remove, and squeeze out excess solvent. Repeat treatment twice. Air dry, heat 2 hr in oven at 110°, and store in closed container.

(i) *Trichloroethylene.*—Practical grade.

(j) *γ-BHC std soln.*—40 mmg/ml. Dissolve 100 mg γ-BHC (lindane) in 100 ml HOAc. Dil. 4 ml aliquot to 100 ml with HOAc.

(Reference grade lindane is available from Nutritional Biochemical Corp., 21010 Miles Ave., Cleveland 28, Ohio.)

24.093 PREPARATION OF STANDARD CURVE

(Following directions refer to application of Beckman Model DU spectrophotometer, using 1 cm cell, where linear relationship is obtained over range 0–200 mmg. Range may be modified, depending on instrument and cell length employed.)

To reaction flasks add 0, 1, 2, 3, 4, and 5 ml of the std γ-BHC soln and dil. to 15 ml with HOAc.

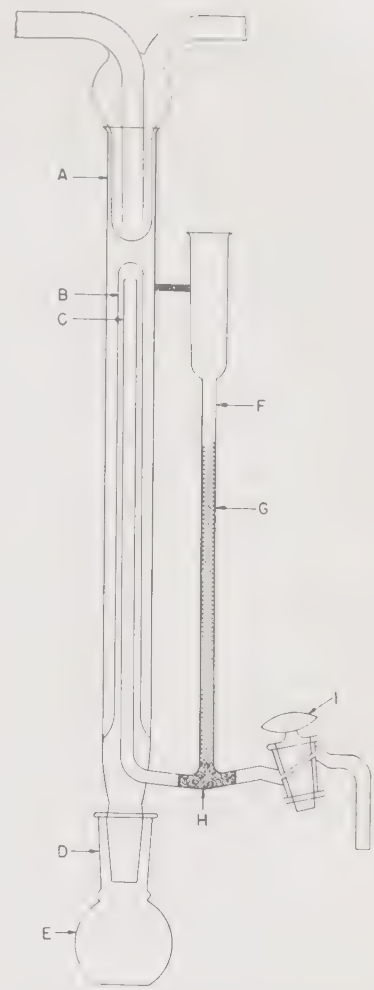

FIG. 49.—BENZENE HEXACHLORIDE APPARATUS

Add 1.5 g powd. Zn and 2 g malonic acid thru funnel, making sure that no particles adhere to neck of flask. Lubricate ground joint and stopcock of app. with H_3PO_4, but do not let any H_3PO_4 fall into reaction mixt. Add 5 ml of the nitrating acid to nitrating tube. Fill outer jacket of app. to ca ¼ mark with trichloroethylene and add small quantity of granular Zn for smooth boiling. Attach reaction flask to app., apply heat cautiously at first, and then boil vigorously at least 2.5 hr. Vapors of trichloroethylene should just reach cold finger condenser. (Trichloroethylene serves to condense HOAc and prevent benzene from condensing before it is swept into nitrating tube. Because liquid in flask is under back pressure, care must be taken that no loss of HOAc (and hence benzene) results thru leakage.) Remove heat source and quickly remove reaction flask from app. so that liquid from nitrating tube is not drawn too far into inner tube. (Nitration mixt. may be left overnight at this stage without losses.)

Add 10 ml ice-cold H_2O to 250 ml separator and

drain nitrating acid into this H_2O. Rinse nitrating and inner tube with 50 ml cold H_2O added in 3 portions; then rinse with 50 ml ether in 3 portions; and finally rinse with 50 ml portion of H_2O.

Shake separator vigorously 1 min., let sep., and drain lower acid layer into second 250 ml separator. Ext. acid with 30 ml ether and discard aq. layer. Shake ether in first and second separators successively with single 30 ml portion 2% NaOH soln; repeat with 30 ml satd NaCl soln. Filter ether ext. thru 0.75″ plug of purified cotton, packed in glass gooch holder, into 250 ml g-s. erlenmeyer. Drain ether from second separator into first, using it as rinse, and filter thru the cotton. Then wash separators and cotton with three 15 ml portions ether. Proceed at once with evapn of ether and color development.

Add glass bead and 1 drop mineral oil to combined ether solns and evap. on steam bath to ca 3 ml. Cover steam bath vent and continue evapn over bath, while swirling flask, until vol. is reduced to ca 1 ml. Remove flask from bath and place in warm section of hood until ether is removed *but no longer*. (These precautions are necessary because *m*-dinitrobenzene is somewhat volatile.) Pipet 10.0 ml methyl ethyl ketone into flask and swirl until residue dissolves. Add 1.0 ml 40% KOH soln and shake vigorously at least 1 min.

If absorbances are detd in Coleman or Beckman Model B instruments, transfer contents of flask to matched tube, stopper, and let color develop in dark 20 min. Read absorbances at 565 mμ against blank of 10 ml of the ketone which has been shaken vigorously with 1 ml 40% KOH soln.

If Beckman Model DU spectrophotometer is used, develop color in original flask 20 min. in dark and carefully decant upper ketone layer into absorption cell. Det. absorbance immediately, since color fades when sepd from alkali. (Instruments which require warming up period should be allowed to attain equilibrium before sepg ketone layer and filling absorption cell.) Plot absorbance against mmg γ-BHC.

24.094 PREPARATION OF SAMPLE

(Generally applicable to vegetables and fruits except citrus)

Finely chop ca 1 kg sample or entire contents of small unit pkg in suitable food chopper (powered mechanical chopper such as Hobart is satisfactory) and transfer well-mixed 100 g portion to high speed blender. Add 100 ml H_2O and blend 2 min. Add 100 ml CCl_4 and blend addnl 10 min.

With care to avoid further evapn insofar as possible, transfer mixt. into two 250 ml centrifuge bottles, stopper, and centrifuge 5 min. at 1500 rpm. Pour off upper aq. layer and discard. (Method of performing this step will vary with product. With beans, collards, and apples, CCl_4 may be obtained by puncturing solid pulp layer at 2 opposite points, and withdrawing solvent with rapid-flowing pipet. With sugared strawberries, CCl_4 and lower layer of pulp material form solid mass. Add 50 ml H_2O to each bottle, shake vigorously 2 min., centrifuge, and pour off upper aq. layer. Repeat operation once and add ca 25 g anhyd. Na_2SO_4 to each residue. CCl_4 layer seps from pulp material upon vigorous stirring, and can be withdrawn.)

Transfer CCl_4 (40–50 ml) to g-s. flask, add 5 g anhyd. Na_2SO_4, shake vigorously to dry, filter thru rapid paper, and pipet 10.0 ml into 50 ml beaker. Evap. by gentle heat on steam bath, removing from heat when vol. is reduced to ca 0.5 ml, and complete removal of CCl_4 by swirling warm beaker.

24.095 DETERMINATION

Transfer residue to reaction flask of app. with three 5 ml portions warm HOAc. Cool, and proceed as in **24.093**, converting absorbance to mmg BHC. Calc. to ppm.

Distinction Between Lindane and Technical BHC (*30*)—First Action

24.096 PRINCIPLES

BHC is extd with *n*-hexane. Ext. is refluxed over fuming H_2SO_4 and BHC is extd with acetonitrile. Purified ext. is chromatographed on paper to sep. and identify pesticides.

24.097 REAGENTS

(**a**) *n-Hexane*.—Tech. grade, 95 mole % min., redistd.

(**b**) *Acetonitrile*.—Commercial grade, redistd.

(**c**) *Fuming sulfuric acid*.—30% free SO_3.

(**d**) *Immobile solvent*.—N,N-Dimethylformamide, b.p. 152–154°, 2-phenoxyethanol, m.p. 12–14°, and ethyl ether, 10:2:88 (v/v).

(**e**) *Mobile solvent*.—2,2,4-Trimethylpentane, 99 mole % min.

(**f**) *Chromogenic agent*.—Dissolve 1.7 g $AgNO_3$ in H_2O, add 10 ml 2-phenoxyethanol and 50 ml EtOH, and dil. with H_2O to 200 ml. (If temp. changes cause sepn of 2-phenoxyethanol, add EtOH in 1 ml increments, with mixing, until redissolved.)

(**g**) *Benzene hexachloride std soln*.—Tech. grade (10–15% γ-isomer), 50 mg/10 ml EtOAc.

(**h**) *Lindane std soln*.—99+% γ-isomer, 50 mg/10 ml EtOAc.

(**i**) *Benzene hexachloride-lindane std soln*.—40 mg BHC+10 mg lindane/10 ml EtOAc.

(**j**) *Chromatographic paper*.—Whatman No. 1.

8×8″ sheets. (If paper contains excessive amount of Ag-reacting material, wash thoroly with distd H_2O and dry in air or in oven at 100°.)

24.098 APPARATUS

(**a**) *Chromatographic chamber.*—For ascending chromatography with 8×8″ sheets. Construct tanks, 9″ long×9″ high×3½″ wide, from light stainless steel. From ¼″ strips of the metal, form 2 supports and suspend flush with top from notches on sides at corners of tank. (Arthur H. Thomas Co. No. 3677 has been found suitable.)

(**b**) *Atomizer.*—Sprayer with reservoir calibrated at 10 ml intervals for use with adjustable constant pressure air supply.

(**c**) *Strong ultraviolet light source.*—Such as germicidal lamps (General Electric Co., Cleveland, Ohio), either (1) two 30 watt, 36″ tubes, mounted in reflectors ca 8″ above papers; or (2) two 15 watt, 18″ tubes, mounted in desk lamp fixture ca 4″ above papers.

24.099 DETERMINATION

(**a**) *Extraction.*—Select wt material representative of sample (ca 1000 g, or entire contents of small unit pkg, and comminute in food chopper. Transfer 100 g well-mixed sample to high speed blender with 100 ml H_2O. Add 100 g anhyd. Na_2SO_4 and 200 ml *n*-hexane, line blender cover with Al foil, and blend to uniform slurry; then 5 min. more. Transfer to two 250 ml centrifuge bottles with stoppers covered with Al foil, and centrifuge 5 min. Siphon off as much hexane phase as possible (siphon described in **15.125** is convenient). Puncture caked plant material with thick glass rod at opposite points on bottle wall, and pour off and discard aq. phase. Break up cake with rod, centrifuge, and decant and combine hexane fractions. Add 10 g anhyd. Na_2SO_4, shake, and filter thru rapid paper into graduated cylinder. If total of <100 ml hexane is recovered, discard first ext., take another 100 g portion comminuted product, and repeat from "Transfer 100 g well-mixed sample," increasing amount of solvent so that at least 50% of the hexane is finally recovered.

(**b**) *Purification of extract.*—Transfer total ext., noting vol., to 200 ml 24/40 ₸ erlenmeyer (preferably lipped). Adjust vol. to ca 50 ml by evapg solvent on steam bath with aid of jet of air. Add 10 ml fuming H_2SO_4 (caution!), insert ₸ air condenser, and reflux on steam bath 30 min. Cool, pour mixt. into dry 125 ml separator (lubricate all separators only with *n*-hexane), and drain and discard free-flowing portion of the acid. Decant hexane into another dry reflux app., rinse flask and separator with 5 ml hexane, and add rinsing to reflux app. Add 10 ml fuming H_2SO_4, reflux, sep. acid, and rinse as before, collecting hexane in 125 ml g-s. flask. Add three 100 mg portions filter aid (diatomaceous earth, such as Hyflo Super-Cel), swirling ca 30 sec. after each addn. Filter thru 9 cm acid-resistant paper (Whatman No. 50 or equiv.) into 250 ml separator. Rinse flask twice with 5 ml hexane, and add washings to filter, letting filter drain between addns.

Ext. hexane with four 60 ml portions H_2O, shaking gently to avoid emulsions. Discard aq. phase, leaving any emulsion. Add H_2O to completely fill separator, and drain and discard aq. phase. (If emulsion is present after final washing, add anhyd. Na_2SO_4 in excess of satn, draw off any aq. phase that may have formed, and filter hexane thru rapid paper into 250 ml separator, washing first separator and filter with two 5 ml portions hexane.) Ext. hexane with four 40 ml portions acetonitrile satd with *n*-hexane, shaking vigorously, and combine exts in 250 ml separator. Wash acetonitrile ext. by shaking with 10 ml *n*-hexane.

Evap. acetonitrile ext. portionwise in 100 ml beaker on steam bath with aid of jet of air, maintaining vol. of at least 1 ml. When vol. is 1–2 ml, remove from steam bath, and evap. to dryness with gentle current of air. (Note odor; if tech. BHC is present, its characteristic odor is readily distinguished from that of essentially odorless lindane.) Dissolve residue in 2 ml acetone, and transfer to 10–15 ml conical centrifuge tube graduated in 0.1 ml. Complete transfer with three 1 ml washings of the beaker with acetone. Place tube in H_2O bath at 30–35°, and evap. acetone to 0.5 ml with aid of gentle jet of *dry* air. Stopper tube tightly with glass or Al foil-covered stopper.

(**c**) *Chromatography.*—(*See* **24.100.**) Attach top edge of chromatographic paper with stainless steel spring clips to glass rod from which paper will be suspended in chromatographic chamber. Apply starting spots of stds and residues, alternately, to paper at 0.75″ intervals, 1″ from bottom edge, keeping extreme spots ca 1″ from side edges. With capillary pipet, spot std solns: 3 microliters of (**g**); 1 microliter of (**h**); and 3 microliters of (**i**). Spot aliquots of the acetone soln of purified ext. equiv. to 1 and 3 g of product. (If total BHC content of product is known, **24.095**, spot aliquots equiv. to 10 and 20 mmg BHC.) Note vol. acetone soln remaining after spotting.

Place required amount of mobile solvent in chamber trough so that bottom edge of paper will be immersed 0.25–0.50″ in solvent, and close chamber. Invert paper and clip bottom edge to glass rod in hood. Spray paper uniformly with immobile solvent, immediately place in chamber contg mobile solvent, seal, and let develop. (Development takes ca 50–75 min.) Solvent front must not reach top edge of paper. Remove paper

from chamber and mark solvent front with soft pencil. After trimethylpentane evaps, invert paper and attach to rod in hood. Spray paper with chromogenic agent to thoroly moisten paper and let hang until H_2O has apparently evapd. (Do not let paper dry completely; 2-phenoxyethanol gives paper damp appearance.)

Expose paper to strong ultraviolet light (first back side, then front side) until violet-colored spots are fully defined. (Exposure time of at least 15 min. for each side is usually required.) Identify pesticide by comparing heights of spots obtained from ext. with those obtained from stds. If it is necessary to repeat chromatography, adjust vol. of acetone soln to correct for any evapn, and spot aliquots to obtain spots to approx. intensities of spots obtained from stds.

24.100 TECHNIC

Paper chromatographic techniques of this procedure have been published in detail (*31*). Following notes may be helpful to analysts not generally familiar with paper chromatography:

Avoid contamination of chromatographic paper by (*a*) rejecting first and last sheets in package, (*b*) handling paper only by rod or clips, (*c*) placing paper only on scrupulously clean surfaces (glass, stainless steel, filter paper, etc.), (*d*) using clean rods, clips, ruler, etc., and (*e*) keeping Ag-reacting fumes from paper.

Use hard pencil for ruling starting line, dotting, and identifying initial spots. In spotting, prevent the portion of paper being spotted from making direct contact with bench surface. Keep diam. of initial spots <5 mm by spotting portionwise in presence of stream of air. Spot all sheets with std solns.

Perform all spraying in hood. In spraying, never let phase being applied pile up so that it begins to flow on paper. Apply immobile phase uniformly by spraying horizontally with back-and-forth motion extending just beyond either side edge of paper, beginning with edge of paper attached to rod in hood and continuing down to "top" edge attached to glass rod used for suspension in tank. Practice spraying immobile solvent on blank sheet so that total of ca 20 ml is used. Solvent front resulting from uniformly sprayed paper will be straight horizontal line.

Protect eyes and skin from strong ultraviolet light.

Degree of sepn of isomers, other factors being constant, depends on amount of immobile phase applied. R_F values of isomers vary inversely with amount of immobile phase. Av. R_F values of α, γ, δ, and β-isomers are ca 0.75, 0.60, 0.35, and 0.20, resp. The o,p'- and p,p'-isomers of DDT are carried thru purification procedure, and have R_F values significantly greater than those of BHC isomers.

It is advisable to gain familiarity with paper chromatographic procedures by chromatographing series of stds before sample ext. is chromatographed.

CAPTAN (N-(TRICHLOROMETHYLTHIO)-4-CYCLOHEXENE-1,2-DICARBOXIMIDE) (*32*)—OFFICIAL

(Applicable to firm fruits such as apples, pears, peaches, and plums)

24.101 PRINCIPLES

Captan is stripped from crop with benzene; H_2O, color, and appreciable amounts of waxes are removed, and red color is developed by fusion of the captan with resorcinol at 135°; color changes to yellow on addn of HOAc.

24.102 REAGENTS

(**a**) *Resorcinol.*—Must be free of discoloration and pass following tests: Fuse 0.5 g and dissolve in 25 ml HOAc. Absorbance at 425 mμ is not >0.015, against HOAc. 1.00 g should not lose >2 mg in 4 hr over H_2SO_4; if more is lost, dry over H_2SO_4 until test is satisfactory.

(**b**) *Cleanup mix.*—10 parts Nuchar, 5 parts Hyflo Super-Cel, and 5 parts anhyd. Na_2SO_4.

(**c**) *Captan std soln.*—Transfer 150 mg pure captan (available from California Spray Chemical Corp., Richmond, Calif.) to 50 ml vol. flask and dil. to vol. with benzene. Pipet 10.0 ml aliquot into 100 ml vol. flask and dil. to vol. with benzene. Pipet 10.0 ml of the dil. soln into 100 ml vol. flask and dil. to vol. with benzene. (1 ml = 0.0300 mg captan).

24.103 DETERMINATION

Weigh accurately ca 500 g sample into clean, dry jar with screw cap faced with sheet cork gasket covered with wet filter paper, or other solvent-tight lid, and add 500 ml benzene. Multiples of sample to benzene ratio can be used. Agitate 15 min., and drain benzene into container and transfer to separator. (Transfer to separator may be omitted where there is no separable aq. layer.)

Transfer ca 100 ml sepd benzene layer to 250 ml g-s. flask, and decolorize and dehydrate with 3–4 g cleanup mix, (**b**), by shaking vigorously ca 5 min. Filter thru folded paper, rejecting first 10–15 ml. Pipet 5 ml filtrate into 25×200 mm test tube and add 0.5±0.1 g resorcinol. Heat 20 min. in oil bath at 135±5°, cautiously at first to evap. benzene; then immerse reaction tubes to depth of ca 2″, but do not let them touch bottom of bath. Remove, and immediately add 10–15 ml HOAc,

followed by rapid immersion in H_2O at room temp. Transfer quantitatively to 25 ml vol. flask, using HOAc, dil. to mark with HOAc, and mix.

Det. absorbance at 425 mμ in 1 cm cell against HOAc within 1 hr. Calc. ppm from std curve.

24.104 PREPARATION OF STANDARD CURVE

Prep. std curve simultaneously with samples. Pipet 0, 2, 4, and 5 ml aliquots of the final std soln into 25×200 mm test tubes and add benzene to make total vol. of 5 ml in each tube. Add 0.5±0.1 g resorcinol and continue as in detn, beginning "Heat 20 min. in oil bath . . ."

NOTE.—One drop H_2O in reaction tube will cause apparent loss of ca 20% captan. Do not leave benzene aliquots in unstoppered reaction tubes where condensation of moisture will take place.

DDT (1,1,1-TRICHLORO-2,2-BIS(CHLOROPHENYL)ETHANE)—FIRST ACTION

24.105 PRINCIPLES (*33*)

Commercial DDT consists essentially of two isomers, *p,p'* and *o,p'* DDT, in proportion of ca 3:1. One of two methods presented involves org. chlorine detn; other is colorimetric method.

(a) *Organic chlorine method.*—Pure DDT contains 5 atoms of Cl; 50% of wt of molecule is Cl. This Cl can be converted into inorg. NaCl by action of Na and isopropyl alcohol and titrd with $AgNO_3$. Because Volhard and electrometric methods are not highly sensitive or accurate for small quantities of Cl, at least 0.2 mg DDT (=0.28 ml 0.01*N* $AgNO_3$) must be isolated. DDT can be extd from various kinds of samples by org. solvents (benzene is one of best) and then treated with Na. This reaction is general for org. Cl, and therefore is not specific for DDT. Only when insecticide used is definitely known to have been DDT can Cl values be calcd to DDT. Inorg. Cl must be excluded from reaction or their effects calcd from blanks.

(b) *Colorimetric method.*—The dry isomers of DDT can be nitrated to tetranitro DDT, which can be extd by ether, dried, and taken up in measured vol. benzene. Treating this soln with stdzd anhyd. NaOMe soln produces reasonably stable colors—blue for *p,p'* and reddish-purple for *o,p'* isomer. This reaction (Schechter-Haller) is fairly specific for DDT. Exceptions are nitrated or dehalogenated decomposition products of DDT and certain close analogues that can produce yellow to red and sometimes blue colors with NaOMe. If distinctly "off" colors are produced on samples of unknown spray history and cannot be removed from the solvent by careful washing with alkali, analogues may be present, and special methods for their detection must be applied.

Principal known interfering insecticides are TDE (1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane), methoxychlor (1,1,1-trichloro-2,2-bis(*p*-methoxyphenyl)ethane), DNB and DNP (the nitro 1,1-bis(*p*-chlorophenyl)butane and propane analogues of DDT). Other insecticidal compounds, as well as limited quantity of benzene-sol. plant extractives, are oxidized or degraded in nitration and are then removed from ether or petr. ether soln by washing with alkali, or they do not react under conditions of method. Other org. material does not seriously interfere, but if >100 mg of extraneous org. matter is present, as in fats, special sepns must be made.

General Methods

24.106 PREPARATION OF SAMPLE

(a) *For firm fruits such as apples, pears, peaches and plums, from which DDT residue is efficiently removed by "stripping" (washing DDT from surface, without serious penetration of solvent into interior and without extracting organic matter other than reasonable quantities of coloring matter and plant waxes or fats) by tumbling with benzene in end-over-end shaking device or in revolving apparatus, or by hand shaking in closed jars.*—Weigh 2–3 kg fruit into clean dry jar (ca 3 gallon), so mounted that it can be turned with end-over-end tumbling action by hand crank or motor (*2*). Add 500 ml benzene and stopper with tight-fitting cork, wooden bung, or plastic screw-cap faced with gasket of sheet cork or other suitable solvent-resisting material. (ACS quality benzene is satisfactory for colorimetric method; essentially Cl-free reagent is necessary for org. Cl method. WARNING: As benzene vapors are *toxic*, take precautions against inhaling them at all times.) Turn jar 5 min. at 75–100 rpm. (Since apples treated for longer periods become swollen and soft, limit time of stripping; on firmer products time factor is not so important.) Open jar and drain off benzene as completely as possible into 1 L erlenmeyer. (It is assumed that concn of DDT in benzene poured off from apples is same as that in solvent retained by apples after wetting.)

Aliquots of this soln may be used directly for DDT detns by colorimetric method if they do not contain >100 mg foreign org. matter, or by org. Cl method if provision is made for electrometric titrn and detn of blanks on untreated or control samples (if procurable) from same source. This is important if small quantities of DDT are involved.

Partially remove coloring matter and plant waxes as follows:

Add, to each 100 ml of the benzene soln, 10 g mixt. compounded by wt as follows: 10 parts anhyd. Na_2SO_4, 5 parts Attapulgus clay (Attapulgus Clay Co., 210 W. Washington Sq., Philadelphia 5, Pa.), 5 parts Filter-Cel (Johns Manville, 22 E. 40th St., New York 16, N. Y.), and 2 parts Nuchar. (For org. Cl method, if last three items are not Cl-free, leach with warm dil. HNO_3, wash on filter with H_2O, using suction, and dry.) Stopper flask, shake vigorously 5 min., and filter on folded paper (Cl-free if org. Cl method is to be used). (This operation dehydrates the benzene, promotes rapid filtration, and removes entrained inorg. Cl, coloring matter, and ca half the natural waxes.) Do not allow appreciable evapn of filtrate before detg DDT in aliquot.

(b) *For hay, cured (dry).*—Finely chop 1 kg sample and strip well-mixed 100 g portion with 500 ml benzene as in (a), except increase stripping time to 1 hr and omit the adsorbent treatment.

(c) *For all fresh or frozen vegetables, meat, and canned food except milk, and for all soft or wet materials generally: Beans (green), broccoli, brussels sprouts, cauliflower, cabbage, cherries, cranberries, grapes, lettuce, pea pods, spinach, squash, tomatoes, silage, etc.*—Finely chop 1 kg sample or entire contents of small unit package in suitable food chopper (powered mechanical food chopper such as Hobart is satisfactory) and transfer well-mixed 100 g portion to high speed blender. Add 100 ml isopropyl alcohol and blend 2 min. Add 200 ml benzene and blend again 2 min. (To avoid splashing and possible loss of sample, regulate blending speed with rheostat or autotransformer when necessary.)

Pour mixt. as completely as possible into 600–800 ml beaker and let solids settle. Pour solvent layer equally into two 250 ml centrifuge bottles. With aid of stirring rod and funnel, distribute solid material equally between the 2 bottles. Stopper and centrifuge 5 min. at ca 1500 rpm. If solids pack firmly, decant supernatant directly into 500 ml vol. flask. If solids pack loosely, transfer liquid thru siphon tube, **15.125**, Notes. Wash blender cup, cap, and beaker with two 100 ml portions isopropyl alcohol-benzene (1+2). Distribute washings equally between the 2 bottles. Break up solid material with stirring rod, stopper bottles, and shake vigorously 2 min. Centrifuge as before and add solvent layer to vol. flask. Dil. to vol. with isopropyl alcohol-benzene (1+2). (Final soln should be clear.)

(d) *For flour, cereals, feeding stuffs, or other comparatively fine dry materials.*—Ext. 25 g sample with ether or benzene in Soxhlet app. or shake larger samples with suitable quantities of solvent in centrifuge bottle, centrifuge, and decant solvent. Repeat extn once or twice, according to size sample. If necessary, sep. fat and DDT as in **24.112(b)**, and det. DDT in ext. by colorimetric or org. Cl methods, according to amount of DDT expected.

(e) *For milk, cream, cheese, butter, oils or fats, etc.*—As DDT is dissolved in fat phase of dairy products, first sep. fats; then isolate DDT and det. by colorimetric method (5 g fat is upper limit). Or hydrolyze fat and use org. Cl method. (At least 0.2 mg DDT is required for significant $AgNO_3$ titrn.)

(1) *Organic Cl determination.*—To 200 g or more milk (proportionate quantities of higher fat dairy products, emulsified with 100 ml H_2O if necessary) in separator, add equal vol. alcohol and ext. with 250 ml ether-Skellysolve B (3+1), shaking gently to prevent formation of stable emulsions. After sepn, drain aq. layer and ext. 3 more times with 100 ml portions of the mixed solvent. Shake vigorously 5 min. in each of last 3 extns and discard aq. phase. Combine ether exts in flask and evap. solvent almost to dryness on steam bath. Remove last traces of H_2O by addn and distn of two 50 ml portions benzene.

(2) *Colorimetric determination.—Liquid milk.*—Weigh 100 g samples well-mixed fresh or HCHO-preserved milk into large centrifuge bottles and add 100 ml alcohol. Mix, add 50 ml redistd petr. ether, stopper bottles with clean rubber stoppers, and shake vigorously 1 min. Centrifuge to break emulsions and blow off upper ether layers into sep. 125 ml g-s. erlenmeyers with siphon arrangement similar in principle to that of wash bottle. (*See* **15.125**, Notes. Do not include appreciable amounts of alcohol-serum layer.)

If emulsions do not break readily, add 1–2 drops HCl, shake vigorously, and again centrifuge. Ext. similarly with 3 addnl 50 ml portions petr. ether, combining exts in respective flask by evapg off ether from one extn while subsequent exts are being prepd. Finish evapn with aid of gentle current of air.

Cheese or other high-fat dairy products.—Work quantity of cheese calcd to yield not >5.0 g fat into suspension with ca 100 ml H_2O, add 100 ml alcohol, and ext. with petr. ether as for liquid milk.

Organic Chlorine Method

24.107 REAGENTS

(a) *Silver nitrate soln.*—0.10*N*, 0.05*N*, or 0.01*N*. *See* **42.025** and **42.029**.

(b) *Ammonium or potassium thiocyanate soln.*—0.10*N*, 0.05*N*, or 0.01*N* NH_4CNS or KCNS. *See* **42.002–42.004**.

24.108 DETERMINATION

(a) *"Strip" solns from fruits and forage crops.*—Evap. aliquots of strip soln from fruits, or forage

crops on steam bath until nearly solvent-free, but not to complete dryness (DDT may decompose with loss of HCl). Add 25–50 ml *99% isopropyl alcohol*, then 2.5 g Na (in form of ribbon or cut in small pieces), and shake flask to mix sample thoroly. Connect flask to reflux condenser and boil gently 1 hr with excess Na present at all times, adding more Na if necessary. Shake flask occasionally. Then eliminate excess Na by cautiously adding 10 ml 50% isopropyl alcohol thru condenser at rate of 1–2 drops/sec. Boil addnl 10 min.; then add 100 ml H_2O.

Cool, add 2 or 3 drops phthln, neutralize by adding HNO_3 (1+1) dropwise, and then add 5 ml excess. If soln is colored, cool to room temp., transfer soln and aq. washings to small separator, and shake with 15 ml *isoamyl alcohol-ether* (1+1). Drain aq. layer into second separator and ext. again with 15 ml of the isoamyl alcohol-ether mixt. Drain aq. layer into 250 ml beaker, and wash 2 org. exts successively with two 10 ml portions H_2O. Combine aq. wash solns with aq. soln in beaker. Make slightly alk. with NaOH soln, add 10 ml 30% H_2O_2, heat to boiling 10–15 min., and neutralize with HNO_3 (1+1), adding 5 ml excess. Or, as optional treatment, after adding 100 ml H_2O, transfer to 400 ml beaker, add 10 ml of the H_2O_2, and heat to boiling on steam bath until H_2O_2 decomposes and most of alcohol evaps. Add H_2O to vol. of ca 250 ml, neutralize with HNO_3, and add 3 ml excess. Cool, and filter thru rapid paper, if necessary. (Treatment with the ether-isoamyl alcohol mixt. is generally unnecessary, especially if electrometric titrn is to be used.) Det. Cl by one of following methods:

(1) Add slight excess of std $AgNO_3$ soln (concn according to quantity of DDT expected) and proceed as in **42.029**. Subtract reagent blank and obtain DDT by multiplying Cl value by 2.

(2) Add excess std $AgNO_3$ soln from buret; then add 5 ml *nitrobenzene* and 0.5 g Fe alum, and swirl flask to coagulate ppt. Back-titr. excess $AgNO_3$ with std KCNS soln until faint pink appears. Cross-titr. with both std solns, crossing end point in each direction (end point, not too sharp, is more easily perceived in this way). Calc. DDT as in (1) from quantity of $AgNO_3$ required for titrn.

(3) Cool flask to room temp. and transfer contents (200–350 ml) to 400 ml beaker. Titr. with std $AgNO_3$ soln, using Ag, Ag-AgCl electrodes in electrometric titrator (Fisher Titrimeter or equiv.). Calc. DDT as in (1).

(b) *Benzene extracts containing fats (milk, other dairy products, fats, oils, extracts of fatty plant or animal organs or tissues).*—

(1) Add, to suitable flask, aliquot or entire benzene ext. of fatty samples (up to 20 g fat) and evap. most of benzene, but not to dryness. To residue add 150 ml isopropyl alcohol and ca 6 g Na cut into small pieces. Reflux 2 hr with moderate boiling, with excess Na present at all times. Add 25 ml alcohol and let flask stand few min. to react with any remaining Na. Dil. with 100 ml H_2O poured thru condenser. Transfer liquid to 600 ml beaker and evap. most of it on steam bath. Dil. to ca 400 ml with H_2O (soap must be in soln). Make acid with H_2SO_4 to ppt fatty acids. Cool with ice or tap H_2O and filter. Wash ppt twice with H_2O. Combine aq. filtrate and washings and ext. with two 100 ml portions *isoamyl alcohol-ether* (1+1). Make aq. soln alk. to phthln with 2*N* KOH. If vol. is >ca 300 ml, conc. by evapn. Make soln acid with HNO_3, add slight excess, and det. Cl by titrn with std $AgNO_3$ soln, either electrometrically or as in **42.029.**

(2) Add to Pyrex flask aliquot of the benzene ext. contg not >10 g fat, and distill off benzene to ca 50 ml in flask. Dil. smaller vols to ca 50 ml with benzene. Add 2 g Na freshly cut into ⅛″ cubes; then add 5 ml *99% isopropyl alcohol* and reflux 90 min. Cool, and cautiously add 20 ml H_2O thru condenser. To avoid fires, swirl flask vigorously until *all* Na reacts. (H_2O will not react with Na explosively in presence of benzene, and alcohol is almost without effect.) Cool flask and transfer contents to separator with 10 ml H_2O and two 5 ml portions HNO_3 (1+1). Make combined exts acid to litmus paper with the HNO_3, add 5 ml excess, and cool. Add 15 ml ether to break emulsions. Shake separator vigorously 1 min. and let layers sep. (Fatty acids are dissolved in benzene-ether layer.) Drain aq. phase into 250 ml erlenmeyer thru funnel contg small wad of cotton moistened with H_2O. Repeat extn with three 5 ml portions H_2O. Det. Cl in combined exts as in (a).

Colorimetric Method

24.109 REAGENTS

(a) *Ether.*—Peroxide- and aldehyde-free.

(b) *Redistilled ether-petroleum ether.*—(1+4).

(c) *Benzene.*—Redistd. Distill until no more H_2O comes over, and discard distillate; replace condenser with dry one, and collect balance of distillate.

(d) *Nitrating mixture.*—Mix fuming HNO_3 (sp. gr. 1.49–1.50) with equal vol. H_2SO_4. Chill before using.

(e) *Sodium methylate soln.*—1.74*N*. 40 g Na/L MeOH. Prep. anhyd. MeOH as follows: Place 75 ml "absolute" MeOH in flask provided with reflux condenser and add 5 g clean Mg turnings. Add 0.5 g I and warm gently, if necessary, until vigorous evolution of H sets in; then reflux until most of Mg has been converted to $Mg(OMe)_2$. Add mixt. to ca 900 ml untreated MeOH, reflux 30 min., and distill with exclusion of atmospheric

H_2O. Preserve in tightly stoppered bottles. (Or dehydrate by refluxing 2–4 hr with 25 g/L CaH_2 and then distg.)

Place 20.0 g freshly cut Na in flask provided with reflux condenser and add portionwise thru condenser enough purified MeOH to dissolve all the Na, warming and refluxing if necessary. Rinse into 500 ml vol. flask with more of the purified MeOH, cool, dil. to mark, and mix thoroly. Chill, centrifuge down any carbonate turbidity, and decant clear supernatant into dispensing system that excludes atmospheric H_2O and CO_2. Adjust batches of NaOMe soln to std concn by titrn with std acid, and prep. new std curve for each batch as made.

Store in dispensing system with all outlets trapped against CO_2 and H_2O and with inner delivery tube to buret packed with dry, alkali-treated asbestos to filter soln immediately before use.

(f) *Pure p,p′ (m.p. 108–109°) and o,p′ (m.p. 74–74.5°)-DDT.*—Reference grade isomers and tech. grade mixt. available from Nutritional Biochemical Corp., 21010 Miles Ave., Cleveland 28, Ohio. *p,p′* Isomer may be prepd from 200 g tech. DDT by recrystg 2 or 3 times from alcohol, and *o,p′*-DDT from mother liquors by concn, fractional crystn from *n*-pentane, and recrystn from MeOH. *o,p′*-DDT may be synthesized as in Haller, *et al.*, J. Am. Chem. Soc. **67**, 1591(1945).

(g) *DDT std solns.*—(1) *Synthetic soln.*—Weigh into 2 sep. 50 ml vol. flasks 50 mg of the 2 pure isomers and dil. to mark with benzene. Pipet 15 ml *p,p′* soln and 5 ml *o,p′* soln into 100 ml vol. flask and dil. to mark with benzene. 1 ml = 0.2 mg total DDT (0.15 mg *p,p′* and 0.05 mg *o,p′*). (2) *Technical soln.*—Prep. soln contg 0.2 mg tech. DDT/ml.

24.110 PREPARATION OF STANDARD CURVES

(a) *For 0–1.0 mg range.*—Measure 0, 1.00, 2.00, 3.00, 4.00, and 5.00 ml of either std DDT soln into 50 ml erlenmeyers and evap. solvent on steam bath. Remove residual vapors with gentle current of air, weight flasks, and immerse in pan of ice-H_2O. (Use pan shelved so that vessels do not rest directly on bottom; porcelain desiccator platform in pan of proper size makes convenient bath.) Thoroly chill flasks and slowly add to each 5 ml chilled nitrating mixt. Rotate flasks to wet all portions of residue; then place pan on hot plate or steam bath and heat so that solns reach ca 85° in 20–30 min. Remove flasks from pan, place directly upon active steam bath, and nitrate 30 min. Remove flasks and cool under tap or leave overnight.

Slowly pour chilled acid mixt. from each flask into separator contg ca 25 ml ice-cold H_2O, rinse flask with several portions of ice-H_2O, and pour rinsings into separator. Rinse again with 25 ml ether-petr. ether (1+4), and finally with second 15 ml portion ether-petr. ether (1+4), pouring last rinse into second separator. Ext. by shaking first separator vigorously 1 min.; then drain aq. layer into second separator and repeat extn. Discard aq. layer and drain second separator into first, rinsing with small portions of ether mixt. Bleed off any residual aq. layer as completely as possible, add 10 ml 10% KOH soln, and shake vigorously 30 sec. Drain off closely and wash with two 15 ml portions satd NaCl soln. Drain well and filter ext. thru 0.5″ layer of washed and dried glass wool previously wetted with ether mixt., held in filter tube, into 125 ml erlenmeyer contg glass bead. Rinse separator and filter with few small portions of ether mixt., evap. off ether on steam bath (gentle current of air speeds evapn and controls bumping), and heat 1 hr at 100°.

Cool flask, making sure interior is thoroly dry, and take up residue with exactly 25 ml redistd benzene. Stopper flask and swirl 1 min. to insure complete soln of residue. (Procedure may be interrupted overnight at this stage.)

Transfer 5.00 ml aliquot to small flask and develop characteristic DDT color by adding exactly 10.0 ml of the NaOMe soln. Mix well, let stand 15 min., and then det. absorbance at 600 mμ in cell of appropriate length against benzene-NaOMe soln (1+2). (Readings in blue at 450 mμ and in green at 510 mμ should also be made to check presence of extraneous yellow and red colors when actual samples are read later.) If instrument used records in terms of transmittance, convert to absorbance (−log T).

Measure all colors of series in same (or similar) cells and plot absorbance against mmg DDT on linear coordinate paper to obtain std curve. Stopper absorption cells with glass covers or stoppers. If test tubes are used, close with clean cork stoppers. Either cells with optically fused ends or cells constructed with alkali-resistant cement may be used, but if cemented cells are used, do not leave alk. solns in cells longer than necessary to make photometric measurements, and clean cells immediately after use.

(b) *For 0–50 mmg range.*—Add 0, 10, 20, 30, 40, and 50 mmg DDT, in benzene soln, to series of 50 ml erlenmeyers, each contg 10 mg oleic acid in benzene soln, and evap. to dryness. (Small Pb rings are convenient for weighting flasks. Oleic acid serves as inert buffer material to prevent loss of micro quantities of DDT.) Nitrate as before and ext. with redistd petr. ether. Wash ext. 3 times with 10% KOH soln, shaking vigorously 2 min. each time. Heat final washed residues 1 hr at 100°, take up in 3.00 ml redistd benzene, and develop color with 6.00 ml of the NaOMe soln. Plot absorbance against mmg DDT as in (a).

24.111 REMOVAL OF COLORING MATTER

(**a**) *Applicable to strip solutions of fruits and extracts of fruits, vegetables, and other low fat products.*—Evap. 200 ml aliquot prepd soln, **24.106**(**c**), or suitable aliquot of **24.106**(**a**), (**b**), or (**d**), just to dryness in 400 ml beaker on steam bath with aid of small air current. Dissolve residue in 10 ml CCl_4 and transfer to column prepd as follows:

Grind 10 g Celite 545 thoroly with 3 ml 15–20% fuming H_2SO_4 in mortar, add 3 ml H_2SO_4, and grind well. Transfer at once to fritted glass funnel or tube (funnel: 40 mm i.d., 60 ml capacity, coarse porosity; tube: 30 mm disk, 130 ml capacity, coarse porosity (sulfur absorption tube, No. K-85900, Kontes Glass Co., Vineland, N.J. is satisfactory)). Pack adsorbent to firm level surface with flat-end glass rod. Add CCl_4 to column to wet adsorbent and let solvent drain until ca 2 mm layer remains on top of column.

Transfer 10 ml CCl_4 sample soln to column. Rinse sample beaker with three 10 ml portions CCl_4 and add rinsings to column. Collect eluate in 125 ml g-s. flask. Let solvent drain until level just reaches surface of adsorbent and rinse walls of tube with 10 ml CCl_4. Repeat with second 10 ml portion CCl_4. After solvent drains to surface level, add 25 ml CCl_4 and let drain completely. At no time permit solvent to sink entirely into column (go dry) until this point is reached. Tamp surface of column with flat-end glass rod to remove all free liquid.

(**b**) *Applicable to extracts of fatty materials containing maximum of 5 g fat.*—Proceed as in (**a**), except use 30 g Celite 545 with 9 ml 15–20% fuming H_2SO_4 and 9 ml H_2SO_4, and 250 ml CCl_4 for elution.

24.112 DETERMINATION

(**a**) *Applicable to strip solutions of fruits and extracts of fruits, vegetables, and other low fat products.*—Evap. solvent just to dryness on steam bath with aid of small air current. Carefully nitrate residue as in **24.110.** To insure thoro removal of interfering oxidation byproducts, wash ether mixt. with six 10 ml portions 10% KOH soln.

If quantity of DDT is unknown, develop exploratory color with 5 ml aliquot and 10 ml NaOMe soln. If absorbance is <0.20, check by taking 10–15 ml aliquot, evapg to dryness, redissolving in 5 ml benzene, and developing color; if absorbance is >0.80, check by taking smaller aliquot, dilg to 5 ml with benzene, and developing color. In all cases, even if exploratory absorbance reading falls in range 0.20–0.80, develop color on another aliquot of nitrated sample as check.

Det. absorbance of final soln at 600 mμ and also take readings at 510 and 450 mμ to det. if gross amounts of interfering red or yellow colors are present. Obtain total DDT in aliquot from std curve and calc. total DDT in sample in ppm.

(**b**) *Applicable to extracts of fatty materials containing maximum of 5 g fat.*—Proceed as in (**a**), except use all of nitrated sample to develop color, and use 0–50 mmg std curve method, **24.110**(**b**).

MALATHION (S-[1,2-BIS(ETHOXYCARBONYL)ETHYL] O,O-DIMETHYL PHOSPHORODITHIOATE) (*34*)—FIRST ACTION

24.113 PRINCIPLES

Malathion is extd with either CCl_4 or mixt. of CCl_4 and isopropyl alcohol and decomposed by alkali in CCl_4-alcohol soln into Na O,O-dimethyl phosphorodithioate, Na fumarate, and alcohol. The Na O,O-dimethyl phosphorodithioate is converted to the cupric salt which is sol. in CCl_4 with formation of intense yellow color. Color intensity is proportional to concn of O,O-dimethyl phosphorodithioic acid and is measured photometrically at 418 mμ.

24.114 REAGENTS

(**a**) *Malathion std soln.*—Dissolve 0.1 g purified material (obtainable from American Cyanamid Co., Stamford, Conn.) in absolute alcohol and dil. to 250 ml with absolute alcohol. Mix well, transfer 25 ml aliquot to 250 ml vol. flask, and dil. to mark with absolute alcohol (1 ml = 40 mmg malathion).

(**b**) *Carbon tetrachloride.*—Reagent grade; or tech. grade distd from all-glass app. at steam bath temp. and stored in amber bottles, which meets following test: Evap. ca 500 ml on steam bath to 100 ml with aid of jet of air. Add known amount of malathion (ca 0.5 mg) in alcohol and det. malathion, using reagent grade CCl_4, as in prepn of std curve. Absorbance found should agree closely with absorbance of same amount of malathion carried thru prepn of std curve, when reagent grade CCl_4 is used.

(**c**) *Carbon disulfide soln.*—Dissolve 1 ml CS_2 in 200 ml reagent grade CCl_4.

(**d**) *Ferric chloride soln.*—Dissolve 5 g $FeCl_3 .6H_2O$ in 100 ml 1*N* HCl.

(**e**) *Copper sulfate soln.*—Dissolve 3.5 g $CuSO_4 .5H_2O$ in 100 ml H_2O.

(**f**) *Sodium sulfate soln.*—Dissolve 90 g anhyd. Na_2SO_4 in H_2O and dil. to 1 L.

24.115 PREPARATION OF SAMPLE

Where possible, use sample size and vol. CCl_4 such that 100 ml of the CCl_4 ext. contains 0.1–1.0 mg malathion. To det. 0.2 ppm malathion, use

vol. ext. equiv. to at least 500 g sample. Conc. larger vol. CCl_4 to 100 ml by evapn on steam bath with aid of jet of air.

(a) *For firm fruits such as apples, pears, peaches, and plums.*—Prep. as in **24.106(a)**, substituting CCl_4 for benzene as solvent, and strip 10 min. Do not use heat to dry wet samples before extn and do not treat with adsorbent. Filter CCl_4 strip solns to obtain clear H_2O-free soln.

(b) *For fresh or frozen vegetables and soft or wet materials generally.*—Finely chop ca 1 kg sample or entire contents of small unit package in suitable food chopper (powered mechanical chopper, such as Hobart, is satisfactory) and transfer well-mixed 100 g portion to high speed blender. Add 100 ml isopropyl alcohol and blend 2 min. Add 200 ml CCl_4 and blend addnl 2 min. Centrifuge, and pour CCl_4-isopropyl alcohol layer into separator. Wash with four 50 ml portions H_2O to remove isopropyl alcohol (if emulsions occur, use 2% aq. Na_2SO_4 soln for washing). Use 100 ml washed CCl_4 ext. for detn.

24.116 PREPARATION OF STANDARD CURVE

Pipet 0, 2.5, 5, 10, 15, 20, and 25 ml aliquots std soln, **24.114(a)**, into sep. 250 ml separators contg 100 ml CCl_4 and 1 ml CS_2 reagent, **24.114(c)**, and add absolute alcohol until total vol. of alcohol is 25 ml. Mix by gentle swirling, add 75 ml Na_2SO_4 soln, **24.114(f)**, which has been acidified with 2.5 ml HCl, and shake vigorously 1 min. Let layers sep. and filter CCl_4 layer thru 12 cm fluted paper into dry 250 ml separator. Do not let any of aq. layer run into filter paper and do not wash paper. Add 25 ml absolute alcohol to separator contg filtered CCl_4 soln, and mix by swirling. Add 1.0 ml 6*N* NaOH and shake exactly 1 min. Immediately add 75 ml of the Na_2SO_4 soln cooled to 15° and shake vigorously 1 min. Let sep.; drain CCl_4 layer and discard it.

Add 25 ml CCl_4 to separator, shake vigorously 30 sec., let sep., and discard CCl_4 layer. Add to separator 25 ml CCl_4, 2 drops phthln, and 6*N* HCl, dropwise with swirling until pink disappears; then add 1 ml 5% $FeCl_3$ soln, **24.114(d)**. Shake vigorously 30 sec., let sep., and discard CCl_4 layer. Again add 25 ml CCl_4, shake vigorously 30 sec., let sep., and discard CCl_4 layer. Repeat extn of aq. layer third time with 25 ml CCl_4 and discard it. Pipet in 25 ml CCl_4 and 1 ml $CuSO_4$ soln, **24.114(e)**; shake vigorously exactly 1 min. and let sep. Immediately filter CCl_4 layer thru small plug of cotton, placed loosely in funnel stem, into 1 cm cell. Det. absorbance of the yellow soln (stable only 5–10 min.) at 418 mμ, against reagent grade CCl_4. Prep. std curve by plotting absorbance of each aliquot against mmg malathion.

24.117 DETERMINATION

Transfer 100 ml of the CCl_4 ext. contg not >1.0 mg malathion, to 250 ml separator; add 1 ml CS_2 reagent **24.114(c)**, and 25 ml absolute alcohol; and mix well by swirling. Proceed as in prepn of std curve, beginning " . . . add 75 ml Na_2SO_4 soln, **24.114(f)** . . . ", discarding small amount of emulsion layer and suspended solids that may have formed after shaking with $FeCl_3$ soln. After this extn, repeat extn of aq. phase, using 25 ml portions CCl_4 until colorless (measure absorbance at 418 mμ). After final CCl_4 extn, drain CCl_4 as completely as possible and discard.

To aq. phase in separator add by pipet 25 ml CCl_4 and then 1 ml of the $CuSO_4$ soln. Immediately shake 1 min. and let sep. Immediately filter CCl_4 layer thru small plug of cotton, placed loosely in funnel stem, into 1 cm cell. Measure absorbance of yellow soln (stable only 5–10 min.) at 418 mμ, against reagent grade CCl_4.

From std curve read amount of malathion corresponding to the absorbance and calc. ppm malathion in sample. Ppm malathion in sample = mmg malathion found/g sample in aliquot analyzed. Det. crop blank by carrying same amount of malathion-free crop thru method.

NOTE: For apples use 1 ml 5% CS_2 in CCl_4 in place of 1 ml 0.5% CS_2 reagent used for other products. In subsequent neutralization of alk. aq. layer with HCl solns, flocculent white ppt forms. Retain ppt with aq. layer when CCl_4 washings are drained. On final washing drain small amount of suspended solids at CCl_4-aq. layer interface.

METHOXYCHLOR (1,1,1-TRICHLORO-2,2-BIS(*p*-METHOXYPHENYL)-ETHANE) (*35*)—FIRST ACTION

(Applicable to firm fruits such as apples, pears, peaches, and plums)

24.118 PRINCIPLES

Petr. ether soln of ext. of sample is extd with acetonitrile to sep. methoxychlor from fats and plant waxes. Methoxychlor in the concd ext. is dehalogenated and converted by alk. hydrolysis to the dehydrochloride which is sepd by petr. ether extn and reacted with 85% H_2SO_4 to produce a red color.

24.119 REAGENTS

(a) *Acetonitrile.*—B.p. 80–82°.

(b) *Petroleum ether.*—B.p. 30–60°. If off colors due to charring by H_2SO_4 are produced in color reaction, purify petr. ether by distn and passing thru column of magnesia and Celite (1+1).

(c) *Alcoholic potassium hydroxide soln.*—Dissolve 20 g KOH in alcohol and dil. to 1 L with alcohol.

(d) *Sulfuric acid with ferric chloride.*—Cautiously mix 900 ml H_2SO_4 with 225 ml H_2O contg 15–20 mg $FeCl_3.6H_2O$. Concn of the H_2SO_4 is critical in development of color and should fall within 84–86% H_2SO_4. Store in Pyrex automatic buret.

(e) *Methoxychlor std soln.*—Dissolve 0.1 g *p,p'*-methoxychlor, m.p. 88–89°, in benzene and dil. to 100 ml with benzene (1 ml = 1 mg methoxychlor). Dil. 1 ml of this soln to 100 ml with benzene (1 ml = 10 mmg methoxychlor). (Reference grade material available from Nutritional Biochemical Corp., 21010 Miles Ave., Cleveland 28, Ohio.)

24.120 PREPARATION OF SAMPLE

Prep. as in **24.106**(a). Finally dil. portion of benzene wash soln with benzene in g-s. graduated cylinder so that 1 ml is equiv. to 1 g of washed product.

24.121 DETERMINATION

Transfer 5 ml of the dild benzene wash soln to 50 ml beaker and evap. to dryness with gentle current of air. Dissolve residue in 20 ml petr. ether and transfer soln to 125 ml separator thru small pledget of cotton in funnel. Rinse with 20 ml and 10 ml petr. ether. Ext. with 15 ml acetonitrile, shaking vigorously 2 min. Let sep. and drain lower acetonitrile layer into clean separator. Re-ext. petr. ether with 3 addnl 15 ml portions acetonitrile and discard petr. ether. Shake combined acetonitrile exts with 20 ml petr. ether and drain acetonitrile into 125 ml beaker flask (conical flask with pouring spout). Wash petr. ether with two 5 ml portions acetonitrile and combine acetonitrile with that in flask. Discard petr. ether. Heat flask gently on steam bath, introducing gentle current of air into flask, and conc. to ca 5 ml. Avoid evapn to dryness.

Add 50 ml alc. KOH, cover with watch glass, and simmer on steam bath 20–30 min. Cool, and transfer to 250 ml separator, completing transfer first with 25 ml H_2O and finally with 50 ml petr. ether. Shake vigorously 3 min. and let layers sep. Drain lower alk. layer into another separator contg 35 ml petr. ether. Shake vigorously 2 min. Discard lower alk. layer and combine petr. ether exts in first separator. Wash combined petr. ether exts by shaking ca 30 sec. with 20 ml 50% alcohol. Discard lower alcohol layer, dry stem of funnel with cotton swab, and drain petr. ether into 125 ml g-s. erlenmeyer. Complete transfer by rinsing separator with 10–20 ml petr. ether. Carefully evap. petr. ether soln on hot H_2O bath to ca 5 ml, remove flask, and evap. to dryness by blowing gentle stream of filtered air into flask, using rubber tube with attached small bore glass tube extending into flask.

To residue in flask add exactly 25.0 ml 85% H_2SO_4 reagent, **24.119**(d), and stopper flask immediately. Let stand at least 45 min. with frequent shaking. Det. absorbance at 550 mμ against the 85% H_2SO_4 reagent.

24.122 PREPARATION OF STANDARD CURVE

Prep. reagent blanks and series of at least 3 stds in range 0–150 mmg. Hydrolyze and proceed as in detn, except that acetonitrile isolation is unnecessary. Read at 550 mμ against the H_2SO_4 reagent, and plot std curve.

MONOFLUOROACETIC ACID (SODIUM SALT, "1080") (*36*)

Qualitative Test—First Action

24.123 REAGENTS

(a) *Decolorizing carbon.* *See* **15.009**(b).

(b) *Thiosalicyclic acid soln.*—Dissolve 300 mg thiosalicyclic acid (Eastman's tech. grade is suitable) in mixt. of 2 ml 1*N* NaOH and 18 ml H_2O.

(c) *Potassium ferricyanide soln.*—Dissolve 1 g $K_3Fe(CN)_6$ in H_2O and dil. to 50 ml with H_2O.

24.124 TEST

Prep. sample and ext. as in **24.128–24.129.** If convenient, ext. large enough sample to obtain 2–10 mg 1080. With very low levels of 1080, *e.g.*, 1–5 ppm, ext. large enough sample to obtain at least 0.5 mg 1080.

Sep. ether ext. from any aq. sludge which may have been carried over in extn, add ca 5 g anhyd. Na_2SO_4 and 0.5 g decolorizing C/100 ml ether, and shake vigorously. Let stand ca 15 min. at room temp. with occasional shaking, and decant thru fluted paper into separator. Add ca 25 ml H_2O and enough NaOH soln (ca 1*N*) to make aq. layer alk. after vigorous shaking (outside test paper). Drain aq. layer into 125 ml erlenmeyer and aerate to remove dissolved ether. Using pH test paper and ca 1*N* solns of H_2SO_4 and NaOH, adjust to pH 4–6. Add 0.5 g C and place on steam bath for 15 min.

Cool under tap and filter thru fluted paper into test tube ca 25 mm × 150 mm. Add 1 ml of the thiosalicyclic acid soln and 2 drops NaOH (1 + 1), and mix. Conc. soln to small vol. by placing on steam bath under gentle current of air. Completely dry residue in oven at 130° or, if time is not factor, in 100° oven. (When convenient, overnight drying is satisfactory, with or without prior concn of soln.)

Dissolve *thoroly* dry residue in 2–3 ml H_2O, add 1 ml of the $K_3Fe(CN)_6$ soln, and mix. Appearance of red color is positive test for 1080. Red ppt

forms at once when 1 mg or more of 1080 is present, or upon standing when only fraction of mg is present.

Employ chromatographic instead of C purification in following cases:

(1) With pineapple juice when <2 mg 1080 can be extd.

(2) With grape juice even when 2 mg or more of 1080 can be conveniently extd.

(3) With any food or material when 1080 is strongly suspected and negative test is obtained, using C purification technic.

For chromatographic purification, follow **24.130** for sepg 1080 from other acids. Discard forerun which may contain HOAc and other extraneous materials. Collect percolate fraction large enough to contain all the 1080 as detd by preliminary detn. Ext. fluoroacetic acid from chromatographic percolate with 25 ml H_2O and enough alkali to cause aq. layer to retain alky after vigorous shaking (outside test paper). Drain org. layer and discard. Drain aq. layer into 125 ml erlenmeyer and aerate to remove $CHCl_3$. Pour soln into test tube and continue as above, beginning "Add 1 ml of the thiosalicyclic acid soln . . . "

Quantitative Method—Official

24.125 PRINCIPLES

After suitable sample prepn, the acid is extd with ether and sepd from inorg. fluorides (partially ether-sol.) by partition chromatography on silicic acid using $0.5N$ H_2SO_4 as immobile solvent and $CHCl_3$ contg 10% *tert*-amyl alcohol or *n*-butyl alcohol as mobile solvent. Monofluoroacetic acid in eluate is converted to its Na salt, and quantity is estimated by micro F detn, **24.029**(a), **24.030**, and **24.031**.

24.126 APPARATUS

(a) *Chromatographic tubes.*—18 mm o.d.×250 mm long, prepd from Pyrex tubing.

(b) *Pressure source.*—Compressed air or cylinder of N or CO_2, and means of keeping pressure constant, such as Hg column or diaphragm-type pressure regulator.

(c) *Mixer.*—High speed blender.

24.127 REAGENTS

(a) *Silicic acid.*—Mallinckrodt analytical reagent grade pptd powder, or equiv.

(b) *Mobile solvent.*—Add 100 ml *tert*-amyl alcohol or *n*-butyl alcohol to 900 ml $CHCl_3$, and mix.

(c) *Phosphotungstic acid soln.*—Dissolve 20 g in H_2O and dil. to 100 ml.

24.128 PREPARATION OF SAMPLE

This will vary with type of material. Dissolve sugars in H_2O, acidify with H_2SO_4, and ext. directly. Following procedures for different type materials will be suggestive. Simple H_2O wash may be adequate to prove contamination of certain foods.

(a) *Sugar.*—Dissolve 100 g sample in enough H_2O to give ca 350 ml.

(b) *Flour.*—Place 100 g sample in mixer, add 400 ml H_2O and 5 g pancreatin, and comminute ca 2 min. Adjust to pH 7–8, using satd $Na_3PO_4 .12H_2O$ soln and suitable indicator paper. Transfer comminuted material to tared 1 L erlenmeyer, washing mixer 3 times with 25 ml portions H_2O. Incubate mixt. at 35–40° at least 3 hr. Add 5 ml H_2SO_4 (1+1) and swirl. Add 20 ml of the phosphotungstic acid soln and swirl again. Make to 750 g with H_2O, stopper, and shake vigorously ca 2 min. Filter thru fluted paper or with suction thru büchner (16 cm size is convenient). Or, more quickly, centrifuge and decant supernatant. Use at least 375 g aliquot of filtrate. (Since sp. gr. of filtrate is very close to 1, measuring out aliquot in graduated cylinder is satisfactory.)

(c) *Wheat.*—Grind sample finely in suitable mill, such as Wiley mill. Proceed as in (b).

(d) *Corn meal.*—Proceed as in (b), except omit pancreatic digestion.

(e) *Corn.*—Grind sample and proceed as in (d).

(f) *Peanuts.*—Grind sample finely (like peanut butter) and proceed as in (d), except use 100 ml of the phosphotungstic acid soln. If necessary, refilter thru folded paper to remove oil.

(g) *Cheese.*—Proceed as in (d), except use 40 ml of the phosphotungstic acid soln.

(h) *Other foods such as chili peppers, cacao beans, etc.*—Treat in manner similar to one of preceding foods.

(i) *Biological tissue.*—If material is tough or fibrous, grind it twice thru food chopper. (Soft tissues, *e.g.*, brain and liver, need not be ground.) Place 100 g ground tissue in 800 ml beaker, add ca 300 ml H_2O, cover with watch glass, and boil gently ca 30 min. Transfer material to mixer, rinsing beaker with two 25 ml portions H_2O, and comminute thoroly (ca 2 min.). Transfer comminuted material to tared 1 L erlenmeyer, rinsing mixer with two 25 ml portions H_2O. Add 5 ml H_2SO_4 (1+1) and mix. Add enough of the phosphotungstic acid soln (50–75 ml) to ppt all proteins, then H_2O to make 600 g. Shake vigorously ca 2 min., and filter thru fluted paper or with suction thru büchner. If material does not filter rapidly, return mixt. to flask, add ca 10 ml more of the phosphotungstic acid soln, shake vigorously, and refilter.

Alternative procedure.—Place 100 g ground tissue in mixer, add 300 ml H_2O and 15 g pancreatin, and comminute thoroly (ca 2 min.). Ad-

just to ca pH 8 with satd $Na_3PO_4.12H_2O$ soln, using suitable indicator paper. Transfer comminuted material from mixer to tared erlenmeyer, washing mixer with two 25 ml portions H_2O, and incubate ca 3 hr at 35–40°. Ppt proteins and make to wt as directed previously.

24.129 EXTRACTION

Transfer soln (of sugar) or wt-aliquot of protein-free filtrate (of protein-contg materials) to 200 ml continuous extractor, Fig. 26, page 187. (Tube is 115–120 cm long and 33–34 mm o.d.: side arm, attached ca 63 cm from bottom, is 15–16 mm o.d. Inner tube is 12–13 mm o.d. flared at top to ca 25 mm diam. 1.5 L extractors of this type have been used successfully. Extra coarse fritted filter tip on bottom end of inner tube aids in getting smaller droplets of extg solvent.) For each 50 g soln, add 1 ml H_2SO_4 (1+1). Ext. with ether until all fluoroacetic acid has been extd (detd by preliminary experiment; usually 3–4 hr with 400 ml extractor). Transfer ether ext. to separator of appropriate size.

To extn flask add ca 20 ml H_2O, 2 drops phthln, and enough 1.0N NaOH from buret to give strong alk. color of the indicator after swirling. Pour rinse soln into separator and add more alkali until alk. color of indicator persists in aq. phase after vigorous shaking. Record vol. alkali required. Drain aq. layer into 100 ml beaker and wash ether with two 10 ml portions H_2O, rinsing extn flask each time with the H_2O before pouring it into separator. Add washings to beaker. Carefully adjust alky of ext. just to alk. color of phthln with 0.1N H_2SO_4 and NaOH solns. Evap. neutralized ext. to dryness on steam bath (current of air hastens evapn). If during evapn alk. color of indicator should disappear, add just enough 0.1N NaOH to give alk. color again. Do not continue heating after residue is apparently dry. Slightly moist residue is permissible.

24.130 CHROMATOGRAPHY

To 5 g of the silicic acid in mortar add max. quantity of 0.5N H_2SO_4 that it will hold without becoming sticky (50–80% of its wt). Mix well with pestle; then add ca 35 ml of the mobile solvent and work up into smooth slurry. (If SiO_2 agglomerates in solvent, too much H_2SO_4 was used.) Place small cotton plug in bottom of chromatographic tube and pour in slurry, tilting tube slightly to avoid air bubbles. Let silicic acid pack down under 2–10 lbs pressure applied thru gas pressure regulator. When excess solvent has drained thru (column firm and viscous enough to resist pouring when tipped), column is ready for use. In prepg column take care to avoid cracking or drying out of the gel caused by leaving pressure on after column packs down and all solvent sinks into gel.

To dry or slightly moist residue in 100 ml beaker add enough H_2SO_4 (1+1) (ca 18N), usually 0.5–1.0 ml, to give excess of ca 0.25 ml over quantity necessary to convert all salts to free acid, as calcd from amount of 1N NaOH required to neutralize acid extd by the ether. Wet salts *thoroly* with the acid, using small, narrow blade spatula (steel or monel metal) to loosen salts from glass, and using flat-end glass rod to break up solid particles and mix resulting slurry. Add 5–10 g anhyd. granular Na_2SO_4 to take up excess liquid. Stir well with tamping rod, breaking up any lumps. Add 10 ml of the mobile solvent, stir thoroly, and decant solvent carefully onto column.

Catch percolate in graduate. Apply pressure until all solvent sinks into gel; then release pressure. Add 5 ml of the mobile solvent to beaker and again stir thoroly. Decant solvent carefully onto column and, with aid of narrow-blade spatula, transfer bulk of material in beaker, mostly Na_2SO_4, to column. Renew pressure. When solvent passes ca halfway thru the Na_2SO_4, release pressure. Rinse out beaker with addnl 5 ml solvent and transfer to column. After this washing sinks ca halfway into the Na_2SO_4, fill tube with the mobile solvent and complete collection, under pressure, of enough percolate to obtain all monofluoroacetic acid, as detd by test run on the silicic acid used (ca 50 ml). Collect dropwise: 3–4 ml /min. is convenient rate.

Transfer percolate to 125 ml separator; add ca 20 ml H_2O and enough 1.0N NaOH to give alk. color of phthln (phthln is present in percolate and no further addn is required) in aq. phase, after vigorous shaking. Drain aq. layer into 125 ml erlenmeyer and return solvent layer to separator. Wash solvent twice with 10 ml portions H_2O and add washings to erlenmeyer. Aerate soln with current of air to remove traces of $CHCl_3$. (If excess $CHCl_3$ is not removed, excessive Cl may complicate F distn in next step.)

24.131 DETERMINATION

Transfer aq. ext. to Pt dish with little H_2O and mix with ca 20 ml lime suspension, **24.028**(a), evap. to *dryness*, and ash 15–20 min. at 600°. (Little C in ash will not interfere in detn.) Proceed as in **24.029**(a), beginning "When clean ash is obtained . . . " and **24.030**-**24.031** (100 ml Nessler tubes are preferable). Convert F results to fluoroacetic acid (×4.11) or to Na monofluoroacetate (1080) (×5.26) as desired, and correct for aliquot taken, if any, in extn. Ignore vol. occupied by insol. solids.

PARATHION (37)—FIRST ACTION

PRINCIPLES

Parathion is extd with benzene or isopropanol-benzene and the strip soln is clarified. Parathion is brought into aq. soln and simultaneously reduced to its amine with Zn-HCl. The amine is diazotized and coupled with N-(1-naphthyl)-ethylenediamine to form colored compound.

24.132 REAGENTS

(a) *Benzene.*—Redistd. Discard first 5% of distillate (which contains azeotropic H_2O), dry condenser, and resume distn, leaving ca 20% of the benzene in still.

(b) *Adsorbent mixture.*—10 parts anhyd., powd. Na_2SO_4, 5 parts Attaclay, 5 parts Filter-Cel, 2 parts Nuchar (*See* **24.106**(a).

(c) *Parathion std solns.*—*Soln A.*—Weigh exactly 100 mg pure parathion (available from American Cyanamid Co., 1937 W. Main St., Stamford, Conn.) onto watch glass or glass boat, rinse into 100 ml vol. flask, and dil. to vol. with redistd benzene. Store in cold when not in use, and prep. fresh monthly. *Soln B.*—Dil. 5 ml Soln *A* to 250 ml with the redistd benzene. Prep. fresh weekly. CAUTION: *Parathion is extremely poisonous.* Avoid contact with the skin and breathing of vapors.

(d) *Dilute hydrochloric acid.*—Approx. 0.5*N*. Dil. 44.2 ml HCl to 1 L.

(e) *Sodium nitrite soln.*—0.25%. Dissolve 250 mg in H_2O and dil. to 100 ml. Prep. weekly.

(f) *Ammonium sulfamate soln.*—2.5%. Dissolve 1.25 g in H_2O and dil. to 50 ml. Prep. weekly.

(g) *N-(1-naphthyl)-ethylenediamine dihydrochloride soln.*—1.0%. Prep. daily. Weigh 200 mg into beaker and dissolve in 20 ml H_2O added from pipet. Filter into dark bottle.

24.133 APPARATUS

Spectrophotometer or filter photometer.—Max. absorption of developed color is at ca 555 mμ. Use spectrophotometer set at this wavelength or photometer with monochromatic filter centering at approx. this point. With 1 cm cell, absorbance for 100 mmg parathion is ca 0.33. Practical working range for Beckman Model DU spectrophotometer is 0–200 mmg parathion. For more precise results use longer cells and more restricted std ranges.

24.134 PREPARATION OF SAMPLE

(a) *Firm, relatively tough-skinned fruits, such as apples, pears, etc.*—Wash with redistd benzene as in 24.106.

(b) *Soft fruits, such as peaches, plums, tomatoes, berries, etc.*—Use 1–2 kg sample with 300–500 ml of the redistd benzene, and strip by shaking gently by hand for 5 min. in suitable size jar.

(c) *Fresh, leafy vegetables, such as cabbage, lettuce, greens, etc.*—Finely chop ca 1 kg sample or entire contents of small unit package in suitable food chopper (powered mechanical chopper such as Hobart is satisfactory) and transfer well-mixed 100–200 g portion to high speed blender. Add equal wt H_2O and blend ca 2 min. Add vol. of the redistd benzene equal in ml to wt sample in g, and blend 4–5 min. in covered blender. Pour into large centrifuge bottles, stopper, and centrifuge ca 5 min. If emulsion has not broken, stir in 50 g powd. anhyd. Na_2SO_4 and recentrifuge. (Sometimes aq. layer, overlaid with emulsion, forms at bottom of centrifuge bottle; if so, siphon off aq. layer, mix more of the anhyd. Na_2SO_4 into emulsion, and recentrifuge.) Or:

Blend 100 g sample with 100 ml isopropyl alcohol 2 min. in high speed blender. Add 200 ml benzene and blend again 2 min. Pour mixt. into centrifuge bottles and centrifuge ca 5 min. Transfer supernatants to 1 L separator. Wash blender with two 50 ml portions benzene and transfer to centrifuge bottles. Break up solids with stirring rod, stopper, and shake vigorously ca 2 min. Centrifuge as before and add solvent layer to separator. Wash ext. with ca equal vol. H_2O to remove isopropyl alcohol. Dry benzene layer with ca 30 g anhyd. Na_2SO_4, and dil. to suitable vol. with benzene.

(d) *Other products.*—Handle in manner similar to (c). Grind dried products, such as alfalfa, dried leaves, etc., in Wiley mill and ext. with benzene in large Soxhlet-type extractor 1–2 hr. Satisfactory extn may be achieved in many cases by letting ground material steep overnight in stoppered jars with measured vol. redistd benzene.

In all cases, treat the benzene ext. by shaking 5 min. with absorbent mixt., **24.132**(b) in proportion of ca 10 g to 100 ml ext. Finally, filter thru rapid, folded paper.

24.135 PREPARATION OF STANDARD CURVE

For 0–200 mmg range, add 2.0, 4.0, 6.0, 8.0, and 10.0 ml parathion std Soln *B*, **24.132**(c), to series of 250 ml, 24/40 ₸ erlenmeyers, preferably lipped. Add redistd benzene to 25 ml total vol., and provide blank flask with 25 ml benzene. Add 20 ml of the 0.5*N* HCl and ca 200 mg *finely powd. Zn dust.* Connect to condenser by all-glass adapter fitted with thermometer, place on hot plate at medium heat, and rapidly distill off the benzene. (Vapor temp. is ca 70°; ebullition stops after benzene is eliminated, and vapor temp. falls.) Do not let aq. soln boil; at this point, disconnect flask, add

10 ml alcohol, and reconnect to reflux condenser. Reflux 5 min., remove from hot plate, and cool flask under tap. Treat flasks in succession (time can be saved by distg the benzene from one flask while preceding one is refluxing).

Add ca 100 mg Filter-Cel to each flask and filter into 50 ml vol. flasks thru 9 cm quant. papers (Whatman No. 44, or equiv.). Use long, thin stirring rods in transfer of solns and washings to papers and take care not to exceed vol. of 45 ml in flasks. (CAUTION: Vol. wash H_2O that may be used is limited to ca 15 ml and must be used judiciously if flask and filter are to be properly washed. Let filter drain thoroly between addns. Use small jets of H_2O to rinse down interior walls of flask, and let final portions of rinse H_2O drip over upper portions of paper.)

Add 1 ml of the $NaNO_2$ soln to each flask, mix, and let stand 10 min. Add 1 ml of the NH_4 sulfamate soln to each flask, mix, and let stand 10 min. Then add 2.0 ml reagent (g), dil. to vol., mix, and let stand 10 min. before reading in spectrophotometer at 555 mμ. Colors should be stable at least 1 hr.

Read colors in succession, using "blank" std as reference, and plot absorbances against mmg parathion to obtain std curve. (Absorbance of "blank" std should not change appreciably during course of 1 day but it is well to check it at intervals against H_2O. With 1 cm cells it should read ca 0.017; this figure may vary for different batches of reagent (**g**).)

24.136 DETERMINATION

Place aliquot of clarified soln obtained in **24.134** in 250 ml ₮ erlenmeyer. (Aliquot size, usually 10–200 ml, will depend on wt/vol. relationship of sample and solvent, upon possible interference from excess unremoved waxy materials in case of large aliquots, and upon expected residue content.) If aliquot <25 ml is taken, dil. to 25 ml with the redistd benzene. Add 20 ml of the 0.5*N* HCl and 200 mg finely powd. Zn dust, and proceed as in **24.135.**

Some azeotropic H_2O distills with the benzene; ignore this amount for 25 ml aliquot, but add 4, 8, and 16 ml H_2O to erlenmeyer (in addn to the 20 ml 0.5*N* HCl) for 50, 100, and 200 ml aliquots, resp.

After reduction, chill *thoroly* under tap, add the Filter-Cel, and swirl vigorously to coagulate unremoved waxy material. Let stand few min. with occasional swirling and filter into 50 ml vol. flask. If filtrate is not perfectly bright and colorless, repeat with smaller aliquot.

Develop color, read absorbance against std "blank," and det. mmg parathion from std curve. From wt/vol. relationship of original sample and vol. benzene used as extractant, calc. parathion content of sample in ppm.

PIPERONYL BUTOXIDE (*38*)— FIRST ACTION

(Applicable to wheat)

PRINCIPLES

Strong H_2SO_4 liberates HCHO which is detd colorimetrically with chromotropic acid.

24.137 REAGENTS

(**a**) *Chromotropic acid reagent.*—Dissolve 100 mg Na 1,8-dihydroxynaphthalene-3,6-disulfonate /ml of H_2O, filter, and keep in dark. Prep. daily. (1 ml required for each detn.)

(**b**) *Dilute sulfuric acid.*—Mix carefully 5 vols H_2SO_4 with 3 vols H_2O. Cool to room temp. and store in tightly g-s. container.

(**c**) *Methanolic potassium hydroxide.*—Dissolve 1.4 g KOH in 5 ml H_2O and add 95 ml MeOH (HCHO free).

(**d**) *Methanol.*—If necessary, purify as follows: Reflux 1 L MeOH 1 hr with ca 10 g powd. Al and ca 10 g NaOH and distill ca 800–900 ml.

(**e**) *Hexane.*—Redistd.

(**f**) *Chloroform.*—Reagent or redistd (for wheat extraction).

24.138 PREPARATION OF STANDARD CURVE

Dissolve 0.1000 g piperonyl butoxide in 100 ml benzene. Dil. 10 ml of this soln to 100 ml with benzene. Dil. 20 ml dild soln to 100 ml with benzene (1 ml = 20 mmg piperonyl butoxide).

Add 0, 20, 40, 60, 80, and 100 mmg piperonyl butoxide, resp., to each of 6 g-s. test tubes (15×150 mm) (25–50 ml g-s. centrifuge tubes are also satisfactory) and evap. on steam bath with small jet of air. Evap. last 1–2 ml benzene without heat.

Into each of tubes pipet both 1 ml chromotropic acid reagent and 5 ml of the dil. H_2SO_4. Stopper loosely and place tubes in beaker of boiling H_2O 45 min., remove, and cool in beaker of cold H_2O. When cool, pipet 5 ml H_2O into each test tube, mix well, and read absorbance in spectrophotometer set at 575 mμ against reagent blank prepd similarly. Plot mmg piperonyl butoxide against absorbance.

24.139 DETERMINATION

Ext. sample as in **24.106**, using $CHCl_3$. Evap. with current of air 25 ml (or suitable size aliquot) ext. in small beaker just to dryness. Add 5 ml methanolic KOH. Warm gently just enough to melt wax (do not boil). Let stand 30 min., swirling vigorously at ca 10 min. intervals. Transfer to small separator, rinse beaker with two 5 ml portions

of H_2O, and add to separator. Add 15 ml hexane to separator, shake vigorously 1 min., and let sep. Drain aq. layer and discard. Transfer hexane layer quantitatively to g-s. test tube or centrifuge tube and evap. to dryness with jet of air. Small amount of heat may be used, but evap. last 1–2 ml with air alone. Warmth of hand at this point is sufficient.

Into dried residue pipet both 1 ml chromotropic acid reagent and 5 ml of the dil. H_2SO_4. Swirl vigorously to insure that reagent contacts all of sample and place test tube in boiling H_2O bath. Stopper tube, lightly at first and then tighten. After 45 min. in H_2O bath, remove, and cool to room temp. in beaker of cold H_2O. Pipet in 5 ml H_2O, mix well, and measure absorbance in spectrophotometer at 575 mμ against reagent blank prepd similarly. From std curve calc. piperonyl butoxide in aliquot.

SULPHENONE (*p*-CHLOROPHENYL PHENYL SULFONE) (*39*)—FIRST ACTION

24.140 PRINCIPLES

Tech. grade Sulphenone contains ca 80% *p*-chlorophenyl phenyl sulfone (*p*-ClDPS); the remainder consists almost entirely of related sulfones, bis(*p*-chlorophenyl) sulfone (*p,p'*-ClDPS) and diphenyl sulfone (DPS).

Hexane "strip" solns are extd with acetonitrile which seps Sulphenone components from bulk of waxes, coloring matter, and to some extent from other pesticides. The acetonitrile residue is chromatographed on Magnesol column with hexane-anhyd. ether. The residue of fraction contg *p*-ClDPS is dissolved in iso-octane for detn of ultraviolet absorbance.

Magnesol column seps *p,p'*-ClDPS, *p*-ClDPS, and DPS in that order. DDT, TDE, methoxychlor, and other highly absorbing pesticides are eluted within threshold volume for *p*-ClDPS. A few pesticides (phenothiazine and malathion), when present in amounts well over their tolerances, show slight interference.

24.141 APPARATUS

(a) *Chromatographic tube.*—Std wall Pyrex glass tubing 2.5 cm o.d.×60 cm long ending in ca 45° taper sealed to stopcock with 2 mm bore. Seal medium porosity fritted disk in place just above taper. Lubricate stopcock with starch-glycerol gel or glycerol.

(b) *Pressure source.*—*See* **4.164**(a). Three-way stopcock between column and pressure source permits convenient release of pressure. Connect to column with rubber stopper but avoid wetting stopper with solvents.

(c) *Solvent evaporator.*—Bent glass tubes in support, connected to filtered air source.

(d) *Spectrophotometer.*—Suitable for measuring absorption in ultraviolet between 230 and 260 mμ; with 2 matched, 1 cm absorption cells.

(e) *Glassware.*—Avoid contamination, taking special care with flasks for receiving chromatographic fractions. Rinse successively in small groups with acetone and follow with not <3 rinsings with hexane or iso-octane, according to use. All acetone must be removed. Clean heavily contaminated glassware with chromic acid cleaning soln followed by thoro rinsing and drying.

24.142 REAGENTS

(a) *Hexane.*—B.p. 60–70° (Skellysolve B or equiv.).

(b) *Iso-octane.*—Pure grade, 2,2,4-trimethylpentane (Phillips Petroleum Co., Bartlesville, Okla., or Enjay Co., Inc., New York, N. Y.). Absorbance at 230, 240, and 250 mμ against H_2O should be not >0.100; otherwise purify by shaking 15 min. with 5 g silica gel (28–200 mesh) for each 100 ml in g-s. bottle. Keep in dark; filter when needed. Sample and std absorbances are detd against iso-octane blank. Solvent in both cells must be from same batch of filtered solvent.

(c) *Ethyl ether, anhydrous.*—(Mallinckrodt A.R. or equiv.). Evap. 20 ml at 40–50° and dissolve residue in 20 ml iso-octane. Absorbances at 230, 240, and 250 mμ against iso-octane should be not >0.020. Differential absorbance, $A_{240} - 0.5(A_{230} + A_{250})$ should be not $> \pm 0.003$.

(d) *Hexane-ether solvent mixture.*—Distill hexane, rejecting first 5% and last 10%. Mix 700 ml hexane with 300 ml anhyd. ether. Store in g-s. bottle.

(e) *Acetonitrile.*—Practical grade.

(f) *Magnesol.*—Industrial regular grade (Westvaco Chlor-Alkali Division, Food Machinery and Chemical Corp., New York, N.Y.). Mix batch by tumbling in large jar. Keep in small, tightly-closed containers.

(g) *Sulphenone std soln.*—0.5 mg/ml. Dissolve 50 mg tech. grade Sulphenone (Stauffer Chemical Co., Richmond Research Lab., Richmond 4, Calif.) in ca 60 ml hexane in 100 ml vol. flask on steam bath, cool, and dil. to vol.

(h) *p-Chlorophenyl phenyl sulfone (p-ClDPS).*—Suitable for std (Stauffer Chemical Co.).

24.143 STANDARDIZATION OF CHROMATOGRAPHIC COLUMN

Pipet 1 ml (0.5 mg) Sulphenone std soln into 125 ml Phillips conical beaker and evap. to dryness with gentle air current. Add 5 ml hexane-ether solvent to residue. Form close-fitting metal foil cover over top of beaker, warm to ca 45°,

swirl to insure soln, and set aside while prepg chromatographic column.

Clamp chromatographic tube in ring stand, in vertical position, and place 100 ml graduated cylinder under outlet. Pour 25 ml hexane-ether solvent into tube, add 15.0 g Magnesol thru powder funnel, and wash down tube walls with 50 ml of the hexane-ether. Stir thoroly with glass rod until no more bubbles rise and mixt. is uniform; then remove rod and let settle ca 1 min. Loosen clamp holding tube and tap end on folded towel while slowly rotating tube back and forth, to achieve evenly-packed and smooth-surface adsorbent bed.

Replace tube in stand, clamping near surface of Magnesol to minimize motion at this point, and force solvent into adsorbent with ca 1–2 lb pressure. (When liquids are being forced into adsorbent, release pressure when level approaches adsorbent surface and let flow continue by gravity until liquid layer has practically disappeared. Have filled pipet ready and make next addn to column. Except as otherwise indicated, rinsing of column or transfer of liquids to column is always by pipet with tip circling in contact with tube near top. If vol. delivered is 10 ml or more, add first few ml slowly, as full flow may impair adsorbent surface. Maintain contact of tip with tube to avoid dripping on adsorbent.)

Rinse down column walls with 10 ml hexane-ether solvent and force into adsorbent. Transfer the prepd Sulphenone soln to column with 2 ml pipet. Rinse down walls of conical beaker with 3 ml hexane-ether solvent from pipet and transfer to column. Repeat rinsing with second 3 ml portion of solvent and then force combined soln and rinsings into adsorbent. Rinse column with 10 ml hexane-ether solvent, and force into adsorbent. Replace 100 ml cylinder with 10 ml graduated cylinder. Transfer 10–20 ml hexane-ether solvent to column, and slowly pour in ca 200 ml more. Apply 1–2 lb pressure to maintain elution rate of ca 5 ml/min. Collect twenty 10 ml fractions, alternating two 10 ml graduated cylinders. Pour fractions and drain well into clean 50 ml erlenmeyers, numbered consecutively. After 20th fraction is collected, release pressure and close stopcock. Reserve column for collection of addnl fractions, if required. All fractions should be collected same day.

Evap. the 20 fractions, and also 10 ml hexane-ether solvent for reference, to dryness with gentle air current in shallow H_2O bath at 40–45°. Add 10 ml iso-octane to each flask and swirl at intervals during ca 10 min. to insure complete soln of residue.

Det. absorbances of initial fractions (Note 1) at 248 mμ (*p,p'*-ClDPS max.) and 242 mμ (*p*-ClDPS max.) and locate leading fraction for *p*-ClDPS (Note 2). The sulfones are eluted in following order: *p,p'*-ClDPS, *p*-ClDPS, DPS. In satisfactory column, threshold vol. for *p*-ClDPS will usually be ca 80–100 ml and eluant volume ca 80–100 ml also. If threshold volume is too low, sepn from other pesticides and *p,p'*-ClDPS may not be satisfactory. In such case, prep. new column, using more Magnesol, and recalibrate. If threshold vol. is high, recalibrate, if desired, using less Magnesol. If column is satisfactory, det. absorbances of remaining fractions contg *p*-ClDPS. Det. absorbances of trailing fractions at 242 and 234 mμ (DPS max.) to locate final fraction for *p*-ClDPS.

NOTE 1.—First few fractions normally show little absorbance; considerable absorbance in these fractions indicates absorbing impurities in the Magnesol. Prewashing columns with addnl 50 or 100 ml hexane-ether solvent may suffice to remove impurities. All subsequent columns should then be prewashed with equal quantity of solvent mixt.

NOTE 2.—A slight overlap in elution patterns of *p,p'*-ClDPS and *p*-ClDPS generally occurs, i.e., absorbance of ca 0.050 for min. in-between fraction. Recovery of *p*-ClDPS is not materially affected.

24.144 PREPARATION OF SAMPLE

(Applicable to apples, peaches, and pears)

Proceed as in **24.106(a)**, substituting hexane for benzene as solvent, and strip 15 min. To minimize breakdown of fruit, choose sample or jar of such size as to allow limited movement of fruit. Filter ext. thru folded paper and keep in tightly closed container.

24.145 DETERMINATION OF ABSORBANCES OF STANDARD

Dissolve 100 mg *p*-ClDPS, accurately weighed, in ca 60 ml iso-octane in 100 ml vol. flask on steam bath. Cool, and dil. to vol. Prep. diln contg 1 mg/100 ml and det. absorbances at 230, 240, and 250 mμ against iso-octane. Refill reference and std cell, repeat readings at above wavelengths, and obtain av. reading for each wavelength.

Also det. absorbances at enough points between 220 and 260 mμ to establish absorbance-wavelength curve.

24.146 SEPARATION AND DETERMINATION OF *p*-ClDPS

Pipet aliquot of strip soln equiv. to ca 50–70 g fruit into 125 ml separator and dil. to 25 ml with hexane. (Lubricate stopcock with film of silicone or petrolatum.) Add 10 ml acetonitrile, shake ca 1 min., let sep., and drain into second separator.

Repeat extn with 2 addnl 10 ml portions acetonitrile and discard hexane. Shake combined acetonitrile ext. well with 5 ml hexane and drain lower layer into 125 ml Phillips beaker. Ext. hexane with 5 ml acetonitrile and drain into Phillips beaker. Evap. acetonitrile on steam bath with air current to ca 5–10 ml. Complete evapn in shallow H_2O bath at 40–45° with gentle air current, leaving in H_2O bath ca 3–5 min. after solvent evaps.

Add 5 ml hexane-ether solvent to sample residue and prep. as in **24.143.** Follow with chromatographic sepn of *p*-ClDPS and collect, in graduated cylinder, eluate found in calibration to contain *p*-ClDPS. Transfer to 125 ml g-s. flask, rinse cylinder with small portions of the mixed solvent, and evap. on steam bath with air current to ca 10–15 ml. Complete evapn in shallow H_2O bath at 40–45° with gentle air current, leaving in H_2O bath ca 3–5 min. after evapn of solvent. Pipet 50 ml iso-octane into flask, stopper, and let stand ca 10 min., rotating frequently to insure soln. Det. absorbances, A for sample, A' for std, at 230, 240, and 250 mμ against iso-octane. Calc., for sample, corrected absorbance, $A_{samp.} = A_{240} - 0.5(A_{230} + A_{250})$. Calc., for std, corrected absorbance, $A'_{std} = A'_{240} - 0.5(A'_{230} + A'_{250})$.

Ppm Sulphenone $= (C \times 50/W) \times (A_{samp.}/A'_{std})$, where C = concn of std in mg/ml, W = wt fruit represented by aliquot of strip soln in kg, and 50 = diln factor. If diln is necessary to obtain readings, multiply by this addnl diln factor.

Det. absorbances at enough points between 220 and 260 mμ to det. shape of curve.

THIRAM (TETRAMETHYLTHIURAM DISULFIDE) (*40*)—FIRST ACTION

24.147 PRINCIPLES

Thiram is extd from sample with $CHCl_3$. Treatment with solid CuI results in formation of brown, $CHCl_3$-sol. Cu dimethyldithiocarbamate, and its absorbance is measured at 440 mμ. Other commonly used pesticides do not interfere, with exception of metal dithiocarbamates sol. in $CHCl_3$, such as ferbam or ziram. Moderate amounts of color, waxes, and other extd plant matter do not interfere.

24.148 REAGENTS

(a) *Chloroform.*—Either reagent or tech. grade may be used.

(b) *Thiram std soln.*—Dissolve 50.0 mg thiram (available from E. I. du Pont de Nemours & Co., Wilmington, Del.) in $CHCl_3$ and dil. to 100 ml with $CHCl_3$. Dil. 5 ml of this soln to 100 ml with $CHCl_3$. (1 ml = 25 mmg thiram.)

(c) *Cuprous iodide.*—If not available, prep. as follows: To soln of 10 g $CuSO_4.5H_2O$ in ca 100 ml H_2O, slowly add excess of KI soln. Remove liberated I by adding $Na_2S_2O_3$ soln in slight excess. Filter and wash thoroly with H_2O and with alcohol. Dry at room temp. and crush to fine powder.

24.149 APPARATUS

(a) *Spectrophotometer.*—Suitable for measuring absorbance at 440 mμ.

(b) *Glassware.*—Avoid contamination by rinsing with $CHCl_3$ and drying before use. Rinse app. that may have contained CuI from previous detns with dil. acid, H_2O, alcohol, and $CHCl_3$.

24.150 PREPARATION OF STANDARD CURVE

(To minimize errors due to evapn of solvent, keep flasks closed as much as possible, and cover funnels with watch glasses during filtrations.)

Using buret, add 2.0, 5.0, 10.0, and 15.0 ml dild std soln to 25 ml vol. flasks. Dil. to mark with $CHCl_3$, and mix. Solns contain 2, 5, 10, and 15 mmg thiram/ml, resp.

Transfer ca 10 ml portions of std solns to 125 ml g-s. erlenmeyers, add 10 mg CuI to each, stopper, and let stand 1 hr with occasional mixing. Filter, using 9 cm quant. paper, and read absorbance at 440 mμ against $CHCl_3$ as reference. Plot absorbances against thiram concn in mmg/ml.

24.151 ISOLATION

Corn.—Ext. 200 g by shaking with 100 ml $CHCl_3$ 5 min. in 500 ml g-s. erlenmeyer. Decant ext. thru small funnel (to retain corn kernels) into flask.

Apples.—Weigh 2–3 kg into clean, dry jar (ca 3 gallon). Add 500 ml $CHCl_3$ and stopper with tight-fitting cork, wooden bung, or plastic screw-cap faced with gasket of sheet cork or other suitable solvent-resisting material. Ext. 5 min. by tumbling or other agitation. Decant ext. into flask.

Add anhyd. Na_2SO_4, ca 5 g/100 ml, to decanted ext. Stopper flask, shake 5 min., and filter thru folded Whatman No. 12 or equiv. paper.

24.152 DETERMINATION

(Thiram in $CHCl_3$ soln, particularly in presence of plant extractives, may decompose. Make detns as soon as possible.)

Transfer ca 10 ml filtered ext. to g-s. erlenmeyer and develop color as in **24.150** beginning "Add 10 mg CuI . . ." As reference, use another portion of filtered ext., untreated with CuI. From std curve obtain thiram concn in mmg/ml. If developed color is too intense, dil. with $CHCl_3$, making similar diln of reference ext., and multiply thiram value found by appropriate diln factor.

Ppm thiram = [(mmg thiram/ml) × ml $CHCl_3$ used]/g sample.

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25. Nuts and Nut Products *(1)*

25.001 Preservation of Sample—Procedure

Store sample in air-tight container at 5–10°. Store meats in glass containers only.

25.002 Preparation of Sample—Procedure

(**a**) *Nuts in shell.*—Remove meats from shells, and sep. all shell particles from meats. Skin or spermoderm should be included with meat in all nuts, including peanuts and coconuts unless specifically excluded by description. Prep. sepd meats as in (**b**).

(**b**) *Nut meats, shredded coconut, or small pieces.*—Grind not <250 g twice thru Enterprise No. 5 food chopper, equipped with revolving knife blade and plate with holes ca $\frac{1}{8}''$ diam. (Other types of food choppers, graters, or comminuting devices that give smooth homogeneous paste without loss of oil may be used.) Mix sample well and store in air-tight glass container.

(**c**) *Nut butters and pastes.*—Transfer sample to container of convenient size and shape, warming semi-solid products, and mix carefully with stiff-blade spatula or knife. (Elec. mixers or stirrers may be used instead if sample is of consistency to give uniform mixt.) Store sample in air-tight glass container.

25.003 Moisture *(2)*—First Action

(Not applicable to high sugar products or products contg glycerol or propylene glycol)

Dry sample representing ca 2 g dry material to constant wt (ca 5 hr) at 95–100° under pressure not >100 mm Hg. Report loss in wt as moisture.

25.004 Crude Fat *(3)*—First Action

(**a**) *Direct method.*—If large quantities of sol. carbohydrates interfere with complete extn of fat, ext. with H_2O before making detn. Ext. ca 2 g sample with ether, dried as in **22.032,** 16 hr in Soxhlet-type extractor. Evap. ether, dry residue 30 min. at 95–100°, cool in desiccator, and weigh; continue this alternate drying and weighing at 30 min. intervals to constant wt (1–1.5 hr is usually required).

(**b**) *Indirect method.*—Proceed as in **25.003**; then ext. dried substance 16 hr as in (**a**), and dry as in (**a**). Report loss in wt as ether ext.

25.005 Crude Protein *(4)*—First Action

Det. N as in **2.036,** and multiply result by 6.25. (It may be desirable to defat with petr. ether.)

25.006 Crude Fiber—First Action—*See* 22.040

25.007 Ash *(5)*—First Action—*See* 29.012, or 29.013 if added chlorides are present.

25.008 Reducing Sugars *(5)*—Official—*See* 22.041

25.009 Sucrose *(5)*—Official—*See* 22.042

25.010 Sodium Chloride *(5)*—Official

To 2 g prepd sample, **25.002,** in Pt dish, add and thoroly incorporate 10 ml 10% $Ca(OAc)_2$ soln. For nut butters and pastes, disperse sample in 10 ml acetone before adding $Ca(OAc)_2$, and remove acetone at room temp. with air current. Dry on steam bath, and ash in muffle at lowest visible red heat (550°). (Complete ashing is not necessary.)

Dissolve ash in 25 ml HNO_3 (1+3), add known vol. 0.1N $AgNO_3$ more than enough to ppt all Cl, heat to boiling, cool, add 5 ml Fe indicator, **4.015**(**e**), and titr. excess Ag with 0.1N NH_4SCN, **42.028**(**b**), until permanent light brown color appears. Calc. Cl as NaCl, after correcting for any Cl in the $Ca(OAc)_2$ soln.

25.011 Water-Insoluble Inorganic Residue—First Action—*See* 36.020

PEANUT BUTTER

25.012 Preliminary Examination—Procedure

Make microscopic examination to detect addn of starch or any off-grade material not identifiable chemically.

25.013 Starch *(6)*—First Action

Weigh 4–5 sample by difference into 250 ml centrifuge bottle and ext. twice with 50 ml portions petr. ether, shaking 5 min. each time. Wash down sides of bottle with petr. ether, centrifuge, and pour off solvent, disregarding opalescence. Warm bottle to drive off remaining solvent, and

transfer residue to mortar and grind. Return fine powder to bottle with aid of 100 ml 10% NaCl soln. Shake bottle 15 min., wash down sides with NaCl soln, centrifuge well, and pour off supernatant, disregarding opalescence. Repeat procedure twice.

Ext. in same manner once with 70% alcohol and once with H_2O, shaking 1–2 min. each time. Drain bottle several min., chill, and add from pipet 100 ml HCl (20.5–21.0 g HCl/100 ml) at temp. not >15°. Shake vigorously 3 min., centrifuge well, and pour off soln thru cotton pledget in funnel stem. Cool soln to temp. at which the HCl was added, and pipet off 50 ml into nursing bottle contg 115 ml alcohol. Shake with whirling motion 1 min., let stand 2 min., centrifuge 2 min., pour off thru weighed gooch contg thin asbestos pad, and add 50 ml 70% (v/v) alcohol to ppt. Stopper bottle, shake vigorously, wash down sides with the 70% alcohol, centrifuge lightly, and pour off thru crucible. Repeat once with 70% alcohol and once with alcohol. Dry crucible and contents 1.5 hr at 130° in air, or 5 hr at 98–100° *in vacuo*. Cover crucible, place in desiccator contg efficient desiccant, and weigh when crucible reaches room temp.

SHREDDED COCONUT

25.014 Glycerol—First Action

Ext. 4 times, with suction, 4 g shredded coconut (dried 5–6 hr *in vacuo* at 70°) on filter (fritted glass büchner is most convenient), using for each extn 50 ml petr. ether (b.p. <65°), and allowing 3 min. intervals between extns. Use flat-end glass rod for stirring. After removing fat, ext. residue on filter with four 50 ml portions absolute alcohol, allowing 3 min. intervals with stirring, as before. Dil. ext. to 250 ml with absolute alcohol at room temp.

Pipet 100 ml into 500 ml erlenmeyer, and add 5 ml H_2O and paste made by adding hot H_2O to 2 or 3 g $Ba(OH)_2$ in small mortar. Heat mixt. on steam bath to boiling and boil ca 1 min.; transfer to 250 ml centrifuge bottle and centrifuge at 2000 rpm ca 5 min. Transfer clear liquid to large porcelain dish and wash residue in centrifuge bottle with 50–75 ml absolute alcohol, stirring with glass rod and centrifuging as before. Evap. on steam bath at temp <70° to few drops, or almost dryness.

Transfer to 50 ml g-s. cylinder with 10 ml absolute alcohol and wash dish with two 5 ml portions absolute alcohol. Further wash dish with three 10 ml portions anhyd. ether, shaking g-s. cylinder thoroly after each addn of the anhyd. ether. Transfer to sediment tube and centrifuge 10 min., at 3200 rpm. Transfer clear soln in sediment tube to evapg dish, preferably Pt, and wash sediment tube with 25 ml of mixt. of absolute alcohol and anhyd. ether (2+3), stirring with glass rod and centrifuging as before. Evap. on steam bath at 85–90° to ca 5 ml, add 20 ml H_2O, and evap. to ca 5 ml; repeat this operation twice. Transfer residue with hot H_2O to 50 ml vol. flask and proceed as in **28.075**.

SELECTED REFERENCES

(*1*) J. Assoc. Offic. Agr. Chemists **18,** 419(1935).
(*2*) Ibid. **8,** 295(1925); **31,** 521(1948); **32,** 527 (1949); **33,** 753(1950); **34,** 357(1951); **37,** 845 (1954).
(*3*) Ibid. **31,** 521(1948); **33,** 753(1950); **34,** 357 (1951); **37,** 845(1954).
(*4*) Ibid. **33,** 753(1950); **34,** 357(1951); **37,** 845 (1954).
(*5*) Ibid. **33,** 753(1950); **34,** 357(1951).
(*6*) Ibid. **37,** 845(1954).

26. Oils, Fats, and Waxes

26.001 Preparation of Sample—Procedure

Melt solid fats and filter, using hot H_2O funnel or similar app. Make detns on samples of this melted, homogeneous mass. Filter oils that are not clear. To retard rancidity, keep oils and fats in cool place and protect from light and air.

Moisture and Volatile Matter (*1*)

26.002 *Vacuum Oven Method—Official*

Soften sample if necessary by gentle heat, taking care not to melt it. When soft enough, mix thoroly with mechanical egg beater or other equally effective mechanical mixer.

Weigh 5 $\pm$0.2 g prepd sample into Al moisture dish ca 5 cm diam., and 2 cm deep with tight-fit slip-over cover. Dry to constant wt in vac. oven at uniform temp. 20–25° above b.p. of H_2O at working pressure, which should not be $>$100 mm Hg. Cool sample in efficient desiccator 30 min. and weigh. Constant wt is attained when successive 1 hr drying periods show addnl loss of not $>$0.05%. Report % loss in wt as moisture and volatile matter.

Specific Gravity (Apparent) at 25/25°—Official

26.003 STANDARDIZATION OF PYCNOMETER

Carefully clean pycnometer by filling with satd soln of CrO_3 in H_2SO_4 and letting stand several hr. Empty pycnometer and rinse thoroly with H_2O; fill with recently boiled H_2O previously cooled to ca 20°, and place in constant temp. bath at 25°. After 30 min. adjust H_2O level to proper point on pycnometer and stopper; remove from bath, wipe dry with clean cloth or towel, and weigh. Empty pycnometer, rinse several times with alcohol and then ether, let dry completely, remove ether vapor, and weigh. Det. wt of contained H_2O at 25° by subtracting wt pycnometer from its wt when filled with H_2O.

26.004 DETERMINATION

Fill clean, dry pycnometer with sample previously cooled to ca 20°, place in constant temp. bath 30 min. at 25°, adjust oil level to proper point on pycnometer, and stopper. Remove from bath, wipe dry, and weigh as in **26.003.** Subtract wt empty pycnometer from its wt when filled with oil and divide difference by wt H_2O at 25°, as detd in **26.003.** Quotient is sp. gr. at 25/25° (apparent).

If sp. gr. at 20/20° is required, proceed as above and as in **26.003** but subtract 5° from each temp. specified.

26.005 Temperature Correction for Specific Gravity of Oils (*2*)—Official

If sp. gr. of oil is detd at other than std temp., approx. sp. gr. at 25° may be calcd by following formula:

$$G = G' + 0.00064(T - 25°),$$

where

G = sp. gr. at 25°;

G' = sp. gr. at T/25°;

T = temp. at which sp. gr. was detd;

and

0.00064 = mean correction for 1°.

Index of Refraction—Official

26.006 GENERAL DIRECTIONS

Det. index of refraction with any std instrument, reading oils at 20 or 25° and fats at 40°. Place instrument so that diffused daylight or some form of artificial light can be used for illumination. Circulate stream of constant temp. H_2O thru prisms. Approx. temp. corrections of butyrorefractometer readings may be made by following formula (*3*): $R = R' + K(T' - T)$, where R = reading reduced to std temp., R' = reading obtained at temp. T', T = std temp., and K = 0.55 for fats and 0.58 for oils.

Readings of instruments that give index of refraction directly can be reduced to std temp. by substituting factor 0.00038 for 0.55 in formula. As temp. rises, refractive index falls. Instrument used may be stdzd with H_2O at 20°, theoretical refractive index of H_2O at that temp. being 1.3330. Any correction found should be made on all readings. Index of refraction varies with density and in same direction. If results appear abnormal, compare specific refractive power (*4*) with normal. Calc. specific refractive power from formula $(N-1)/D$, where N = refractive index and D = density. According to Proctor (*5*), Lorenz formula, $(N^2-1)/(N^2+2)D$, gives much more satisfactory results than does $(N-1)/D$.

26.007 *By Means of Abbé Refractometer*

To charge instrument, open double prism by means of screw head and place few drops sample on prism or, if preferred, open prisms slightly by turning screw head and pour few drops sample into funnel-shape aperture between prisms. Close prisms firmly by tightening screw head. Let instrument stand few min. before reading, so that temp. of sample and instrument will be same.

Method of measurement is based upon observation of position of *border line of total reflection* in relation to faces of flint glass prism. Bring this border line into field of vision of telescope by rotating double prism by means of alidade in following manner: Hold sector firmly and move alidade backward or forward until field of vision is divided into light and dark portion. Line dividing these portions is "border line," and, as a rule, will not be sharp line but band of color. Colors are eliminated by rotating screw head of compensator until sharp, colorless line is obtained. Adjust border line so that it falls on point of intersection of cross hairs. Read refractive index of substance directly on scale of sector. Check correctness of instrument as in **26.006**, or with quartz plate that accompanies it, using monobromonaphthalene, and make necessary correction in reading.

26.008 *Butyro-refractometer readings and indices of refraction*

READING	INDEX OF REFRACTION	READING	INDEX OF REFRACTION
40.0	1.4524	60.0	1.4659
40.5	1.4527	60.5	1.4662
41.0	1.4531	61.0	1.4665
41.5	1.4534	61.5	1.4668
42.0	1.4538	62.0	1.4672
42.5	1.4541	62.5	1.4675
43.0	1.4545	63.0	1.4678
43.5	1.4548	63.5	1.4681
44.0	1.4552	64.0	1.4685
44.5	1.4555	64.5	1.4688
45.0	1.4558	65.0	1.4691
45.5	1.4562	65.5	1.4694
46.0	1.4565	66.0	1.4697
46.5	1.4569	66.5	1.4700
47.0	1.4572	67.0	1.4704
47.5	1.4576	67.5	1.4707
48.0	1.4579	68.0	1.4710
48.5	1.4583	68.5	1.4713
49.0	1.4586	69.0	1.4717
49.5	1.4590	69.5	1.4720
50.0	1.4593	70.0	1.4723
50.5	1.4596	70.5	1.4726
51.0	1.4600	71.0	1.4729
51.5	1.4603	71.5	1.4732
52.0	1.4607	72.0	1.4735
52.5	1.4610	72.5	1.4738
53.0	1.4613	73.0	1.4741
53.5	1.4616	73.5	1.4744
54.0	1.4619	74.0	1.4747
54.5	1.4623	74.5	1.4750
55.0	1.4626	75.0	1.4753
55.5	1.4629	75.5	1.4756
56.0	1.4633	76.0	1.4759
56.5	1.4636	76.5	1.4762
57.0	1.4639	77.0	1.4765
57.5	1.4642	77.5	1.4768
58.0	1.4646	78.0	1.4771
58.5	1.4649	78.5	1.4774
59.0	1.4652	79.0	1.4777
59.5	1.4656	79.5	1.4780

26.009 *By Means of Zeiss Butyro-Refractometer*

Place 2 or 3 drops filtered sample on surface of lower prism. Close prisms and adjust mirror until it gives sharpest reading. If reading is indistinct after running constant temp. H_2O thru instrument for some time, sample is unevenly distributed on prism surfaces. As index of refraction is greatly affected by temp., use care to keep temp. constant. Carefully adjust instrument, using std fluid supplied with it. Convert instrument reading to refractive indices from table, **26.008**.

Melting Point of Fats and Fatty Acids—Official

Wiley Method

26.010 REAGENT

Alcohol-water mixture.—Sp. gr. should be same as that of fat to be examined. Prep. by boiling H_2O and alcohol separately 10 min. to remove gases held in soln. While still hot pour the H_2O into test tube until it is almost half full. Nearly fill test tube with the hot alcohol, pouring it down side of inclined tube to avoid too much mixing. If alcohol is added after the H_2O has cooled, air bubbles will make mixt. unfit for use.

26.011 DETERMINATION

Let melted and filtered fat fall 15–20 cm from dropping tube upon piece of ice or upon surface of cold Hg. Disks thus formed should be 1–1.5 cm diam. and should weigh ca 200 mg. Remove disks when solid, and let stand 2–3 hr to obtain normal m.p.

Alternatively, disks may be prepd using app. consisting of Al plate ca 3 mm thick and 100 mm square with perforations ca 10 mm in diam. and steel plate ca 10 mm thick and 150 mm square. Thoroly chill steel plate in refrigerator and place Al plate on top (surfaces should be flush). Pour melted and filtered fat into holes of Al plate and let stand in refrigerator at least 2 hr. Remove fat above surface of Al plate and remove disks.

Place 30×3.5 cm test tube, contg the alcohol-H_2O mixt., in tall 35×10 cm beaker contg ice and H_2O, and leave until mixt. is cold. Drop disk of fat into tube. It will sink immediately to point where density of alcohol-H_2O mixt. is exactly equiv. to its own. Lower accurate thermometer, that can be read to 0.1°, into test tube until bulb is just above disk. To secure even temp. in all parts of alcohol-H_2O mixt. around disk, stir gently with thermometer. Slowly heat H_2O in beaker, constantly stirring with air stream or other suitable device.

When temp. of alcohol-H_2O mixt. rises to ca 6° below m.p. of fat, disk of fat begins to shrivel and gradually rolls up into irregular mass. Lower thermometer until fat particle is even with center of bulb. Rotate thermometer bulb gently and so regulate heat that ca 10 min. is required for last 2° increase in temp. As soon as fat mass becomes spherical, read thermometer. This is Wiley m. p. At this point temp. of bath must be not >1.5° above m. p. of sample. Conduct 2 addnl detns exactly as above. Second and third results should agree closely.

If edge of disk touches side of tube, make new detn.

26.012 *Capillary Tube Method (6)*

Draw ca 10 mm of melted and filtered fat into thin-wall capillary tube, 1 mm i.d. Seal end of tube with sample in small flame. Do not burn fat. Hold tubes contg fat overnight (ca 16 hr) in refrigerator at 4–10°. Attach tube to accurate thermometer graduated to 0.2°, so that lower end is even with bottom of Hg bulb. Suspend in 600 ml beaker half filled with H_2O so that thermometer is immersed ca 30 mm. Starting 8–10° below m. p. of sample apply heat so as to increase bath temp. ca 0.5°/min., agitating H_2O in bath by small stream of air or with slow stirrer. Take as m. p. temp. at which substance becomes transparent. Report av. of 3 detns which should agree within 0.5°.

Titer Test (7)—Official

26.013 SPECIFICATIONS FOR TITER TEST THERMOMETERS

Type.—Etched stem, glass.

Liquid.—Mercury.

Range and subdivision.—Minus 2 to +68° in 0.2°.

Total length.—385–390 mm.

Stem.—Constructed of suitable thermometer tubing of either plain or lens front type. Diam., plain front type: 6–7 mm; diam., lens front type: cross section of stem must be such that it will pass thru 8 mm ring gauge but not enter 5 mm slot gauge.

Bulb.—Corning normal or equally suitable thermometric glass. Length, 15–25 mm; diam., 5.5 mm to not greater than that of stem.

Distance from bottom of bulb to −2° mark.—50–60 mm.

Distance to 68° mark from top of thermometer.—20–35 mm.

Length of unchanged capillary.—Between highest graduation and expansion chamber, 10 mm.

Expansion chamber.—To permit heating to at least 85°. Space above Hg to be evacuated or filled with N or other suitable gas.

Top finish.—Glass ring.

Graduation.—All lines, figures, and letters to be clear-cut and distinct. Each degree mark to be longer than remaining lines. Graduations to be numbered at zero and at each multiple of 2°.

Immersion.—45 mm.

Marking.—"A.O.A.C. Titer Test," serial number, and manufacturer's name or trademark must be etched on stem. Words "45 mm immersion" must also be etched on stem, as well as line extending around stem 45 mm above bottom of bulb.

Scale error.—Error at any point on scale must not be >0.2°.

Standardization.—Thermometer must be stdzd at ice point and at intervals of ca 20°, for condition of 45 mm immersion, and for av. stem temp. of emergent Hg column of 25°.

Case.—Thermometer must be supplied in suitable case on which appears markings "A.O.A.C. Titer Test," "−2° to +68° in 0.2°."

NOTE: For interpreting these specifications, following definitions apply:

Total length is over-all length of finished instrument.

Diam. is that measured with ring gauge or micrometer.

Length of bulb is distance from bottom to beginning of enamel backing.

Top of thermometer is top of finished instrument.

26.014 APPARATUS

Stirring titer assembly, as shown in Fig. 50, consisting of 2 L beaker, wide-mouth bottle (capacity 450 ml, height 190 mm, i.d. of neck, 38 mm), titer test tube (25×100 mm), and stirrer (2–3 mm o.d., one end bent in form of loop, 19 mm diam.).

26.015 DETERMINATION

Heat 110 g *glycerol-KOH soln* (25 g KOH in 125 g glycerol) to 150° in 800 ml beaker and add 50 ml of the oil or melted fat, previously filtered if necessary to remove foreign substances. (Altho saponification often takes place almost immediately, continue heating and frequent stirring 15 min. Do not heat above 150°.)

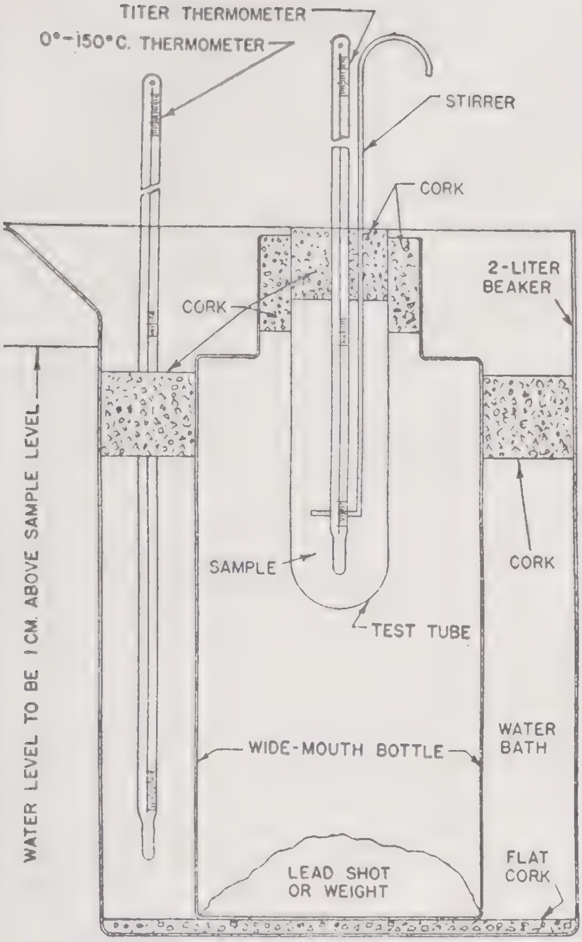


FIG. 50.—TITER STIRRING ASSEMBLY

When saponification is complete, usually indicated by perfectly homogeneous soln, cool slightly and add 200–300 ml H_2O. After complete soln of the soap, add with stirring 50 ml dil. H_2SO_4 (16 ml H_2SO_4 in 70 ml H_2O). Heat soln, with frequent stirring and addn of H_2O if necessary, until layer of fatty acids is completely melted and clear. Siphon off aq. acid layer, add H_2O to fatty acids, boil 2–3 min., and again siphon off aq. layer. Repeat treatment with H_2O until wash H_2O is neutral to Me orange. Remove fatty acids so as not to include H_2O, and filter while melted thru rapid paper. Heat to 130° on hot plate to remove traces of H_2O and pour fatty acids into titer tube to height of 57 mm from bottom. If H_2O is present in fatty acids, decant, refilter, and reheat.

Fill H_2O bath and adjust to 20° for all samples with titers 35° or higher, and to 15–20° below titer for samples with titers $<35°$. (H_2O level should be 1 cm above sample level.) Place test tube contg fatty acids in app., Fig. 50. Insert thermometer to immersion mark and equidistant from sides of tube. Stir vertically with stirring rod at rate of 100 complete up-and-down motions /min., starting agitation while temp. is at least 10° above titer point. (Stirrer should move thru vertical distance of ca 3.8 cm. If preferred, stirring may be performed mechanically.) Continue stirring until temp. remains constant 30 sec. or begins to rise in <30 sec. interval. Discontinue stirring immediately and observe rise in temp. Report as titer highest point reached by thermometer. Duplicate detns should normally agree within 0.2°.

Iodine Absorption Number—Official

(All reports should specify method used)

Hanus Method

26.016 REAGENT

Hanus iodine soln.—Dissolve 13.2 g pure I in 1 L HOAc (99.5%) that shows no reduction with dichromate and H_2SO_4. Add enough Br to double halogen content as detd by titrn (ca 3 ml). The I may be dissolved by heating, but soln should be cold when Br is added.

Convenient procedure for prepg the Hanus I soln is as follows: Measure 825 ml HOAc and dissolve in it with aid of heat 13.615 g I. Cool, and titr. 25 ml of this soln with $0.1N$ $Na_2S_2O_3$, **42.035.** Measure another portion of 200 ml HOAc and add 3 ml Br. To 5 ml of this soln add 10 ml 15% KI soln, and titr. with the $0.1N$ $Na_2S_2O_3$. Calc. quantity of Br soln required to double halogen content of remaining 800 ml I soln as follows:

$A = B/C$, where A = ml Br soln required; $B = 800 \times$ thiosulfate equiv. of 1 ml I soln; and C = thiosulfate equiv. of 1 ml Br soln. If necessary, reduce mixed soln to proper concn by diln with HOAc.

26.017 DETERMINATION

Weigh ca 0.5000 g fat, or 0.2500 g oil (0.1000–0.2000 g of oils that have high absorbent power), into 500 ml g-s. flask or bottle and dissolve in 10 ml $CHCl_3$. With pipet add 25 ml of the Hanus I soln, draining pipet definite time, and let stand 30 min. in dark, shaking occasionally. (This time must be adhered to closely to obtain accurate results. Excess of I should be at least 60% of quantity added.)

Add 10 ml 15% KI soln, shake thoroly, and add 100 ml freshly boiled and cooled H_2O, washing down any free I on stopper. Titr. the I with $0.1N$ $Na_2S_2O_3$, adding it gradually, with constant shaking, until yellow color of soln almost disappears. Add few drops starch indicator, **2.093(d)**, and continue titrn until blue color entirely disappears. Toward end of titrn, stopper bottle and shake violently, so that any I remaining in soln in the $CHCl_3$ may be taken up by the KI soln.

Conduct 2 blank detns along with detn on sample. Number of ml $0.1N$ $Na_2S_2O_3$ required by blank (B) minus no. of ml used in detn (S) gives $Na_2S_2O_3$ equiv. of I absorbed by the fat or oil.

Calc. % by wt of I absorbed (I number, Hanus method).

I number = $[(B-S) \times N \times 12.69]$/g sample, where N is normality of $Na_2S_2O_3$ soln.

Wijs Method

26.018 REAGENTS

Wijs iodine soln.—(1) Dissolve 13 g resublimed I in 1 L HOAc (99.5%), and pass in washed and dried Cl until original $Na_2S_2O_3$ titrn of soln is not quite doubled; or (2) dissolve 16.5 g ICl in 1 L HOAc (do not use ICl_3 to prep. soln (*8*)). Store in amber bottle sealed with paraffin until ready for use. Do not use if >30 days old. Det. I/Cl ratio as follows:

Iodine content.—Pipet 5 ml of the Wijs soln into 500 ml erlenmeyer contg 150 ml satd Cl-H_2O and some glass beads. Shake, heat to boiling, and boil briskly 10 min. Cool, add 30 ml H_2SO_4 (1+49) and 15 ml 15% KI soln, and titr. immediately with 0.1N $Na_2S_2O_3$.

Total halogen content.—Pipet 20 ml Wijs soln into 500 ml erlenmeyer contg 150 ml recently boiled and cooled H_2O and 15 ml 15% KI soln. Titr. immediately with 0.1N $Na_2S_2O_3$.

I/Cl = 2A/(3B − 2A), where A = ml 0.1N $Na_2S_2O_3$ required for I content and B = ml required for total halogen content. I/Cl ratio must be 1.10 ± 0.1.

26.019 DETERMINATION

Weigh 0.1000–0.5000 g (depending on I number; sample wt may be calcd as 25/expected I value) melted and filtered sample into clean, dry, 500 ml g-s. bottle or flask contg 20 ml CCl_4. With pipet add 25 ml of the I soln, draining pipet definite time. Excess of I should be 50–60% of quantity added, that is, 100–150% of quantity absorbed. Swirl, and let bottle stand in dark 30 min. at uniform temp.

Add 20 ml 15% KI soln and 100 ml recently boiled and cooled H_2O. Titr. the I with 0.1N $Na_2S_2O_3$, **42.035**, adding latter gradually, shaking constantly until yellow color of soln almost disappears. Add few drops starch indicator, **2.093(d)**, and continue titrn until blue entirely disappears. Toward end of reaction stopper bottle and shake violently so that any I remaining in soln in the CCl_4 may be taken up by the KI soln.

Conduct 2 detns on blanks in same manner as sample, but without fat. Slight variations in temp. appreciably affect titer of I soln, as HOAc has high coefficient of expansion. It is essential, therefore, that blanks and detns on sample be made at same time. Ml std $Na_2S_2O_3$ soln required by blank (B) minus quantity used in detn (S) gives $Na_2S_2O_3$ equiv. of I absorbed by sample taken. Calc. % by wt of I absorbed as in **26.017** and report as I number, Wijs method.

Thiocyanogen Number—First Action

26.020 REAGENTS

(a) *Lead thiocyanate.*—Dissolve 331 g $Pb(NO_3)_2$ in 700 ml H_2O and filter. Dissolve 194 g KCNS in 500 ml H_2O and filter. Slowly add $Pb(NO_3)_2$ soln to KCNS soln with stirring, continue stirring 30 min., and let ppt settle. Decant supernatant thru filter paper on büchner, using slight suction, and wash ppt several times with H_2O by decantation. Transfer ppt to büchner, using horn spoon and H_2O, and wash with H_2O until washings give no test for nitrates. Place ppt on watch glass and dry to constant wt (ca 7 days) in vac. desiccator over H_2SO_4. Dried $Pb(CNS)_2$ should be white. Store in air-tight brown bottle and keep in dark. Yield, ca 260 g.

(b) *Thiocyanogen soln.*—0.2N. Prep. anhyd. HOAc by boiling gently 500 ml HOAc (at least 99.5%) ca 3 hr with 40 ml Ac_2O in flask with ₮ air condenser. Attach $CaCl_2$ tube to end of condenser and let acid cool to room temp. (Both anhyd. HOAc and Ac_2O must show absence of reducing substances by $KMnO_4$ test.)

Soln 1.—Weigh 8.4 g dry Br into 250 ml vol. flask, dissolve in 100 ml pure dry CCl_4, and dil. to vol. with anhyd. HOAc.

Soln 2.—Pour 250 ml anhyd. HOAc on 25 g of the pure dry $Pb(CNS)_2$ in colorless, dry, g-s. 1 L bottle.

Add Soln 1 to Soln 2 in small quantities, shaking Soln 2 vigorously after each addn and being sure that decoloration takes place before each addn of soln. After complete mixing of Solns 1 and 2, let suspension of pptd $PbBr_2$ and surplus $Pb(CNS)_2$ settle. Filter soln thru dry paper into dry, brown, g-s. bottle. Keep filtrate, which should be clear and colorless, or only slightly yellow, in dark. If correctly prepd, 25 ml of this soln requires 48–52 ml 0.1N $Na_2S_2O_3$, **42.035**, for its iodometric titrn. (The CNS soln will keep ca 1 week. After that time it begins to show yellow color and turbidity, and soon fine yellow ppt settles to bottom of bottle.)

26.021 DETERMINATION

Weigh 0.1–0.7 g fat or oil (excess of CNS reagent should be 150–200%) into 200 ml g-s bottle or flask. Pipet in 25 ml of the SCN soln, rotate bottle gently until fat dissolves, and store in dark 24 hr. Add 10 ml 20% KI soln quickly and at one time, while shaking bottle to avoid hydrolysis of CNS soln. Add 100 ml H_2O and titr. liberated I with stdzd 0.1N $Na_2S_2O_3$ in usual manner, using starch indicator. Conduct at least 2 blank detns along with sample detn. Subtract ml $Na_2S_2O_3$ soln required by sample from ml required by blank and multiply difference by I equiv. of the $Na_2S_2O_3$ soln. Value obtained is quantity of

I equiv. to CNS absorbed by fat or oil. Calc. % by wt and report as CNS number.

When I and CNS values are detd on the oil, calc. % hypothetically pure triglycerides by following formulas:

When no linolenin is present:

$Y = 1.246\ I.V. - 1.253\ T.V.$;
$Z = 2.525\ T.V. - 1.348\ I.V.$;
$S = 100 - (Y + Z)$.

When linolenin is present:

$X = 1.6610\ T.V. - 0.1322\ I.V. + 1.3056\ S - 130.56$;
$Y = 1.4137\ I.V. - 3.3449\ T.V. - 1.6441\ S + 164.41$;
$Z = 1.6839\ T.V. - 1.2805\ I.V. - 0.6615\ S + 66.15$;
X = % linolenic acid glycerides;
Y = % linoleic acid glycerides;
Z = % oleic acid glycerides;
S = % satd acid glycerides and unsaponifiable matter;
$I.V.$ = I number;
$T.V.$ = CNS number.

When I and CNS values are detd on fatty acids, calc. as follows:

When no linolenic acid is present:

$S = 100 - (Y + Z)$;
$Y = 1.194\ I.V. - 1.202\ T.V.$;
$Z = 2.421\ T.V. - 1.293\ I.V.$

When linolenic acid is present:

$X = 1.5902\ T.V. - 0.1290\ I.V. + 1.3040\ S - 130.40$;
$Y = 1.3565\ I.V. - 3.2048\ T.V. - 1.6423\ S + 164.23$;
$Z = 1.6146\ T.V. - 1.2275\ I.V. - 0.6617\ S + 66.17$;
X = % linolenic acid;
Y = % linoleic acid;
Z = % oleic acid;
S = % satd acid;
$I.V.$ = I number;
$T.V.$ = CNS number.

Saponification Number (Koettstorfer Number)—Official

26.022 REAGENT

Alcoholic potassium hydroxide soln (9).—(1) Reflux 1.2 L alcohol 30 min. in distg flask with 10 g KOH and 6 g granulated Al (or Al foil). Distill and collect 1 L after discarding first 50 ml. Dissolve 40 g KOH in this 1 L alcohol, keeping temp. <15° while dissolving alkali. Keep soln in g-s. bottle. Or, (2) crush 40 g KOH in 7 or 8″ mortar. Add 45 g granulated CaO and grind mixt. to powder. From 1 L of alcohol add 100 ml to mortar and transfer to flask, rinsing mortar with several more portions. Add remainder of alcohol to flask, shake mixt. at least 5 min., and invert beaker over neck of flask. Repeat shaking several times during day. Next morning filter soln into clean, dry, g-s. bottle.

26.023 DETERMINATION

Weigh accurately ca 5 g filtered sample into 250–300 ml erlenmeyer. Pipet 50 ml of the alc. KOH soln into flask, draining pipet definite time. Connect flask with air condenser and boil until fat is completely saponified (ca 30 min.). Cool, and titr. with 0.5*N* HCl, **42.009–42.010**, using phthln. Conduct blank detn along with that on sample, using same pipet for measuring KOH soln and draining same length of time. Subtract ml 0.5*N* HCl required in detn on sample from ml required on blank to obtain ml 0.5*N* HCl equiv. to KOH used in saponification of sample taken. Calc. and report as saponification number (mg KOH required to saponify 1 g fat).

26.024 Soluble Acids—Official

Place flask used in **26.023** and its contents on H_2O bath and evap. the alcohol. Add quantity of 0.5*N* HCl equiv. to quantity of KOH used for saponification of sample in **26.023** and 1 ml more (quantity of 0.5*N* HCl to be added = titrn for blank − titrn for sample + 1 ml), and place flask on steam bath until sepd fatty acids form clear layer on upper surface of liquid. Fill flask to neck with hot H_2O and cool contents in ice-H_2O until cake of fatty acids hardens thoroly. Pour liquid contents of flask thru filter into 1 L flask, refill flask with hot H_2O, and set on steam bath until fatty acids collect at surface. Cool by immersing in ice-H_2O and again filter liquid into the 1 L flask. Repeat this treatment with hot H_2O 3 times, cooling and collecting washings in the 1 L flask after each treatment. Titr. combined washings with 0.1*N* NaOH, **42.032–42.033**, using phthln. Subtract 5 (corresponding to excess of 1 ml 0.5*N* acid) from ml 0.1*N* NaOH used and multiply by 0.0088 to obtain wt sol. acids as butyric acid. Calc. % sol. acids.

26.025 Insoluble Acids (Hehner Number)—Official

Let flask contg cake of insol. fatty acids from **26.024** and paper thru which sol. fatty acids have been filtered drain and dry 12 hr. Transfer cake, together with as much of fatty acids as can be removed from filter paper, to weighed, widemouth beaker flask. Place funnel contg filter in neck of flask and wash paper thoroly with hot absolute alcohol. Remove funnel, evap. alcohol, dry 2 hr at 100°, cool in desiccator, and weigh. Again dry 2 hr, cool, and weigh. If there is any considerable decrease in wt, reheat 2 hr, cool, and reweigh. Calc. % insol. fatty acids.

Soluble and Insoluble Volatile Acids (Reichert-Meissl and Polenske Values) (*10*)—Official

26.026 REAGENTS

(a) *Sodium hydroxide soln.*—(1+1). Protect soln from contact with CO_2. Let soln settle and use only clear liquid.

(b) *Silicon carbide.*—Grit No. 6. Carborundum Co., Niagara Falls, N. Y.

(c) *Glycerol-soda soln.*—Add 20 ml of the 1+1 NaOH soln to 180 ml pure glycerol.

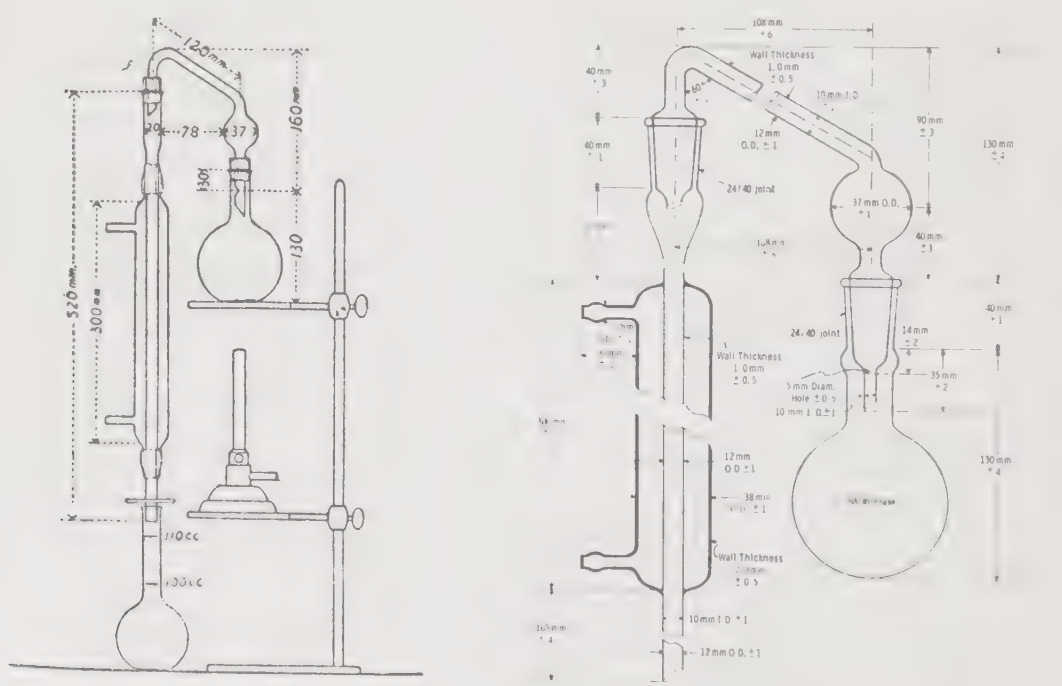
26.027 DETERMINATION

Weigh accurately 5±0.1 g sample into clean, dry 300 ml round-bottom flask. Add 20 ml of the glycerol-soda soln and heat with swirling over flame or asbestos-covered hot plate until completely saponified, as shown by mixt. becoming perfectly clear. No oil should remain on surface; walls of flasks are wet by soln. Let flask cool to ca 100° (ca 5 min.) and *dissolve* contents in 135±1 ml of recently boiled H_2O with min. loss of H_2O vapor. (135 ml H_2O is conveniently measured from 125 ml erlenmeyer previously calibrated to deliver 134.6±1.0 g H_2O at 25°.) Then add 6 ml H_2SO_4 (1+4) and 15 pieces of SiC. Distill, using app. with dimensions given in Fig. 51. (Adapter may be used for distg into 110 ml vol. flask.) Rest flask on piece of asbestos board with center hole 5 cm diam., and regulate flame so as to collect 110 ml distillate in 30±2 min. (measure time from passage of first drop of distillate from condenser to receiving flask), letting distillate drip into receiving flask at temp. of ca 20°.

When 110 ml has distd, substitute 25 ml cylinder for receiving flask, remove flame, and disconnect distn head from condenser. Mix without violent shaking, immerse flask contg distillate almost completely in 15° H_2O 15 min., filter thru dry 9 cm moderately retentive paper (S&S No. 589 White Ribbon is satisfactory), and titr. 100 ml filtrate with 0.1*N* NaOH, **42.032–42.033,** using phthln. Pink color should remain unchanged 2–3 min. Reichert-Meissl value is ml 0.1*N* NaOH used, corrected for titrn obtained in blank detn, ×1.1, calcd to 5.00 g sample.

Remove remainder of sol. acids from insol. acids on filter by washing with three 15 ml portions 15° H_2O, each previously passed thru condenser, 25 ml cylinder, and 110 ml receiving flask. Dissolve insol. acids by passing three 15 ml portions neutral alcohol thru filter paper, each portion having previously passed thru condenser, 25 ml cylinder, and 110 ml receiving flask. Titr. combined alc. washings with 0.1*N* NaOH, using phthln. Polenske value is ml 0.1*N* NaOH required for titrn, after this result is corrected for titrn obtained in blank detn, and calcd to 5.00 g sample.

NOTE.—Unless these directions are followed in every detail, satisfactory results cannot be obtained.



(A) (B)

FIG. 51. APPARATUS FOR DETERMINATION OF REICHERT-MEISSL AND POLENSKE VALUES: (A) WITH RUBBER STOPPERS, (B) WITH GLASS JOINTS

Mole Per Cent Butyric Acid in Fat

Chromatographic Method (11)—Official

26.028 APPARATUS

Chromatographic column.—Fuse 15 cm section of 38 mm o. d. tubing to 20 cm of 22 mm tubing, which in turn is fused to 5 cm of 7 mm tubing, with drip tip.

26.029 REAGENTS

(**a**) *Silicic acid.*—Mallinckrodt No. 2847. Heat in shallow pan or evapg dish 18 hr at 175°. Store in desiccator or tightly sealed container.

(**b**) *Bromocresol green-glycol soln.*—Dissolve 700 mg bromocresol green in 700 ml ethylene glycol by warming on steam bath. Cool, and add ca 200 ml H_2O. Prep. 0.1*N* NH_4OH by dilg ca 6.6 ml NH_4OH to 1 L with H_2O. Add 40 ml of this soln to indicator soln and addnl H_2O to make 1 L. Store this ink-blue soln in stoppered bottle.

(**c**) *Packing material.*—Mix H_2SiO_3, (**a**), in ratio of 100 g to ca 95 ml bromocresol green-glycol soln, (**b**), until homogeneous olive-green powder is obtained. Small batches may be mixed in mortar; larger batches, in mechanical mixer. Prepd packing material may be stored in tightly stoppered container several months.

(**d**) *Hexane-butanol mixt.*—Add 1 vol. *n*-butanol to 100 vols *n*-hexane (commercial grade; Phillips Petroleum Co. or equiv.).

(**e**) *Isopropanol-KOH soln.*—Dissolve 25 g KOH pellets in 400 ml isopropanol by warming and swirling on steam bath. Decant supernatant alc. soln from the small amount of aq. soln clinging to bottom of flask. Cool and decant supernatant isopropanol-KOH soln, which contains ca 50 mg KOH/ml. Store in refrigerator.

(**f**) *Potassium hydroxide soln.*—Approx. 0.05*N*. Dil. 60 ml isopropanol-KOH soln, (**e**), with 440 ml isopropanol and 500 ml methanol. Store in amber or "Life-time Red" Pyrex bottle.

(**g**) *Thymol blue soln.*—Dissolve 300 mg thymol blue in 25 ml 0.05*N* alc. KOH soln, (**f**), and add 75 ml isopropanol.

26.030 PREPARATION OF FATTY ACID SOLUTION

(**a**) *Saponification.*—Transfer 0.5–0.7 g well-mixed, melted fat to 20×150 mm test tube with lip, and add 5 ml isopropanol-KOH soln, (**e**), and boiling chip. Place tube to depth of 2″ in boiling H_2O bath 20 min. to saponify fat, and evap. isopropanol, leaving solid soap. Stopper, and analyze within 48 hr.

(**b**) *Determination of quantity of acid required to hydrolyze soap.*—Add 5 ml isopropanol-KOH soln, (**e**), to 10 ml H_2O and 2 drops thymol blue soln, (**g**), in small beaker or flask. Add H_2SO_4 (2+1) dropwise, with constant stirring, until initial blue color turns to yellow, orange, and finally red. This vol. H_2SO_4, measured with same dropper, is subsequently used to hydrolyze the soap. (If top of chromatographic column turns blue on addn of fatty acid soln, use of more H_2SO_4 to hydrolyze soap is indicated; top of chromatographic column should be yellow.)

(**c**) *Hydrolysis of soap and extraction of fatty acids.*—Add to soap, while cooling tube in cold H_2O, number of drops of the H_2SO_4 (2+1) indicated in (**b**). Break up lumps in bottom of tube with glass stirring rod. After mass in tube is thoroly mixed, yellow mixt. of fatty acids clinging to viscous aq. layer of K_2SO_4 should be obtained. Add 10 ml of the hexane-butanol soln to tube and thoroly mix with glass rod. Aq. phase should cling to white ppt of K_2SO_4, allowing easy decantation of the hexane-butanol soln of fatty acids. This soln of acids is ready for chromatography.

26.031 PREPARATION OF CHROMATOGRAPHIC COLUMN

Overlay 35 g packing material, **26.029**(**c**), with hexane-butanol mixt., **26.029**(**d**), in mortar. Mix with pestle to form slurry. Place small glass wool plug loosely in constricted end of column and gently tamp into place with glass rod. Place finger over constricted end of column and add hexane-butanol mixt. until reservoir is half full. Using teaspoon, underlay prepd slurry beneath solvent. Jiggle spoon up and down along side of reservoir, and let flocculent slurry settle to bottom of column.

After adding all packing material, remove finger and let solvent flow out and packing material settle. Apply 5–10 lb air pressure to top of column to speed up flow of solvent and facilitate uniform packing of slurry. Release pressure just before last portion of solvent sinks into column. If column looks uniformly packed, it is ready for use; if not, add more hexane-butanol soln to reservoir and again apply pressure as before. Prepd column should have flow rate of ca 3.5 ml /min. without use of pressure. If flow rate is <3 ml/min., add more bromocresol green-glycol soln to packing material, and remix. If flow rate is >4 ml/min., add more H_2SiO_3 to packing material, and remix. Hexane-butanol mixt., recovered during prepn of column, may be used subsequently to prep. other columns or for chromatography.

26.032 CHROMATOGRAPHY OF FATTY ACIDS

Decant hexane-butanol soln of fatty acids onto top of packed column and immediately start collecting eluate in 250 ml erlenmeyer. As soon as fatty acid soln completely settles into packing,

wash down inside of reservoir with three 5 ml portions of hexane-butanol mixt. Let each washing sink into packing before refilling reservoir. Yellow band should always be observed at very top of column; this band contains inorg. acids (H_2SO_4 and acid sulfate) and will not move.

If sample contains butter fat, second distinct yellow band due to butyric acid appears and slowly migrates down column. Butyric acid band breaks away from top inorg. acid band. Long-chain fatty acids, C_6 and higher, pass rapidly thru column and do not form yellow bands. There will be eluate vol. of 20–30 ml between elution of last traces of long-chain acids and first traces of butyric acid. When lower edge of yellow butyric acid zone is 1 cm from lower end of chromatographic column, change fraction collector. First fraction contains long-chain acids and usually measures 100 ± 10 ml. Next 120 ml fraction contains butyric acid.

26.033 TITRATION OF FATTY ACID FRACTIONS

Add 1 drop thymol blue soln for each 10 ml eluate being titrd. Titr. each fraction to first permanent appearance of purple-blue end point, using ca 0.05*N* KOH, **26.029(f)**, dispensed from 50 ml buret for first fraction and from 5 ml buret, graduated in 0.01 ml, for second fraction. End point for each fraction is sharp but is subject to fading because of CO_2 absorption. The CO_2 effect is negligible if titrn is conducted rapidly with little agitation. If necessary, titrn may be carried out in CO_2-free atmosphere which tends to make titrn values more reproducible. (Pass air thru 20% aq. KOH soln, then thru H_2O, and finally into titrn flask.)

Blank corrections are not required. Alkali added to thymol blue soln takes care of blank.

Express butyric acid titrn as % of sum of the 2 titrns, calcd to nearest 0.1%. *Example:* Long-chain acid titrn = 26.1 ml; butyric acid titrn = 2.83 ml; sum = 28.93. Mole % butyric acid = $2.83 \times 100/28.9 = 9.8$.

Saturated and Unsaturated Fatty Acids

26.034 *Lead Salt-Ether Method (12)—Official*

(Not applicable to fats and oils that contain erucic, elaeostearic, chaulmoogric, hydnocarpic, or similar acids; to hydrogenated products that contain appreciable quantities of iso-oleic acid; nor to coconut or palm kernel oils that contain appreciable quantities of lower fatty acids that give ether-sol. Pb salts.)

Weigh accurately 10 or 20 g sample into 200 ml erlenmeyer. Add 30 ml alcohol and 8 ml KOH soln (1+1). Mix thoroly and heat on steam bath ca 30 min. Add slight excess of HOAc (1+2), using phthln, and then add enough 15% KOH soln, while rotating flask, to produce distinct pink. Heat to boiling in 1 L flask 60 ml (120 ml for 20 g sample) 20% $Pb(OAc)_2$ soln and same quantity of H_2O. Add the neutralized soap soln cautiously to avoid any loss, rinsing saponification flask with 5 ml alcohol, then with small portions hot H_2O. Boil mixt. gently ca 5 min., shake thoroly, and cool under running H_2O, rotating flask so that all pptd Pb soaps adhere to sides and bottom of flask. When mixt. is cold, pour off aq. soln into large beaker and examine soln for particles of Pb soap. (Usually soln is slightly turbid due to some basic $Pb(OAc)_2$ and no particles or globules of Pb soap are seen.) Wash flask and Pb soap twice with cold H_2O and let flask drain 10 min. Remove last drops of H_2O, using thin roll of filter paper held by forceps, being careful to press paper only lightly against ppt. Add ca 120 ml ether and shake by rotating flask ca 5 min.

Connect flask with reflux condenser and boil gently until Pb soap is completely distintegrated or dissolved. Remove flask and rinse down sides with enough ether to make final vol. ca 150 ml. Invert close-fitting beaker over neck of flask and keep in refrigerator at least 15 hr. Place 7 cm filter paper in 7.5 cm büchner, apply full suction, and fit hardened filter paper cut to 8 cm diam. as snugly as possible to sides of funnel. Decant ether soln from sepd Pb soaps, using only enough suction to draw ether thru filter. (Too much suction causes ether to evap. so rapidly that filter may become clogged with sepd unsatd acids, Pb soaps, or ice.)

Transfer ppt to filter by rinsing flask with small portions of ether. During filtration keep funnel covered as much of time as possible to prevent evapn of ether. If at any time filtration proceeds so fast as to cause mass of Pb soap to crack, close cracks by pressing with small spoon or spatula; otherwise ppt cannot be properly washed. Rinse spoon free of ppt and wash ppt 8 or 10 times with ether, finally letting suction continue until ppt cracks into numerous pieces. Without delay, sep. with spoon as much of ppt as possible and transfer it without loss to 500 ml separator contg ca 50 ml ether, washing off any ppt adhering to spoon and neck of separator with ether. Transfer filter paper to 1 L flask. Shake separator thoroly to disintegrate lumps of Pb salt and let stand ca 20 min.

Add 20 ml HCl previously dild with 10 ml H_2O and shake thoroly 2 min. to decompose all Pb soap. Add 5–10 ml HCl (2+1) to the 1 L flask contg filter paper; shake thoroly to decompose any ppt adhering to flask and filter; then wash into separator with small alternate portions of ether and H_2O until all fatty acids and $PbCl_2$ are removed from flask. Again shake separator with rotary motion and let stand 10 min.

Drain lower aq. soln slowly, taking precautions not to remove any emulsion or undecomposed Pb soap. When Pb soap is present (shown in form of lumps that float on top of aq. soln), add 10 ml HCl and shake again; add ca 20 ml H_2O, shake, and let mixt. stand until layers sep. Drain aq. soln and wash ether with successive 25 ml portions H_2O until washings are HCl-free. Dehydrate ether with ca 2 g anhyd. Na_2SO_4 and transfer soln to weighed 300 ml erlenmeyer. Rinse separator and Na_2SO_4 with several small portions of ether to remove all fatty acids, taking care not to let any of the Na_2SO_4 fall into weighed flask. Distill ether, avoiding any loss of the fatty acids, and heat in oven at ca 110° to constant wt. Obtain wt satd acids and save them for later investigation.

Transfer ether soln of the sol. Pb soaps to 500 or 1000 ml separator, rinsing büchner and filter flask with small quantities of ether. Add mixt. of 30 ml HCl and 75 ml H_2O, and shake with rotary motion 2 min. Let mixt. stand 10 min., then slowly drain aq. soln into beaker. If drops of the ether soln are entrapped by the $PbCl_2$ ppt and are removed with it, decant soln from pptd $PbCl_2$ that has settled into separator. Rinse beaker and ppt with small quantities of ether, adding washings to separator. Rotate contents of separator and let stand 10 min.

Drain aq. soln and wash ether with successive 50 ml portions H_2O until HCl is removed. Transfer ether soln to weighed 300 ml erlenmeyer. Distill ether and place flask in oven heated to ca 110° ca 1 hr, while passing stream of CO_2 into flask to prevent oxidation of unsatd acids. Cool in atmosphere of CO_2. When cold, remove the CO_2 and weigh. Repeat treatment until constant wt is obtained.

Det. in duplicate I numbers of both satd and unsatd acid fractions. (I number of satd acid fraction is due to presence of some unsatd acid.)

To correct for unsatd acids present in fraction of satd acids use following formula:

$$\frac{\text{I No. of satd acid fraction}}{\text{I No. of unsatd acid fraction}} \times 100 = A \text{ (\% unsatd acids in satd acid fraction).}$$

Obtain correct value by formula $A \times B/100$, where B is % impure satd acids (as found by analysis). Subtract this correction from % impure satd acids and add it to % unsatd acids actually detd.

Polyunsaturated Acids (*13*)—First Action

American Oil Chemists' Society Method

26.035 PRINCIPLES

Natural conjugated constituents are detd by measuring ultraviolet absorption at specified wavelengths in purified solvent. Non-conjugated polyunsatd constituents are partially conjugated by heating in KOH-glycol soln and absorptions of conjugated constituents are redetd. The % conjugated diene, triene, tetraene, and pentaene acids are calcd from predetd absorptivities by simultaneous equations.

Method is applicable to detn of polyunsatd acids, dienoic thru pentaenoic, in animal and vegetable fats contg only natural or cis isomers, only small amounts of preformed conjugated material, and only small amounts of pigments whose absorption may undergo considerable change during the alkali isomerization. Method is not applicable, or is applicable only with specific precautions, to hydrogenated oils, or other fats contg trans isomers of unsatd fatty acids, to fish oils or similar fats contg acids more highly unsatd than pentaenoic, to crude oils or samples contg pigments whose absorption undergoes changes during alkali isomerization, or to fats and oils contg large quantities of preformed conjugated fatty acids.

26.036 APPARATUS

(a) *Isomerization apparatus.*—(*See* Fig. 52.) (1) *Constant temperature bath.*—180 ± 1°C. Capacity sufficient to immerse 25 × 250 mm Pyrex test tubes to depth of 4½″ (114 mm). (Westinghouse or equiv. household deepfat fryer has been found satisfactory.) For bath liquid use Fisher Scientific Co. bath wax or DC 550 fluid, Dow Corning Corp., Midland, Mich. Place bath in insulated box with insulated cover having holes for stirrer and cork supports for test tubes.

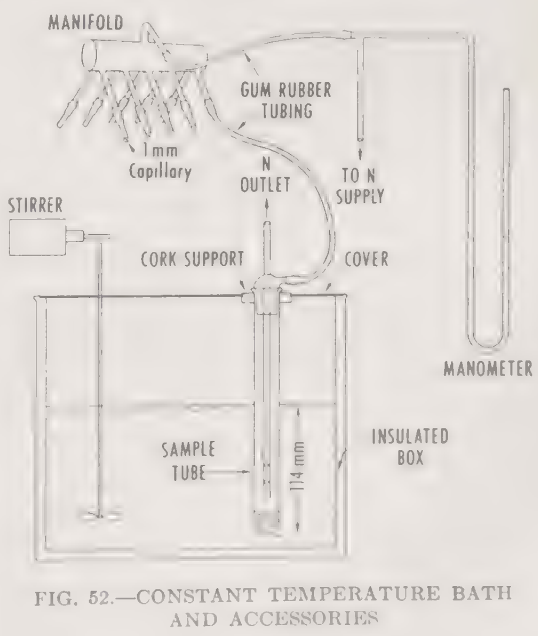


FIG. 52.—CONSTANT TEMPERATURE BATH AND ACCESSORIES

(2) *Test tubes.*—Pyrex, lipped, 25 × 250 mm.

(3) *Distributing heads.*—To fit test tubes snugly. Tubing in center of head has both ends

open and two small holes 25 and 38 mm, resp., from bottom. (*See* Fig. 53, right.)

(4) *Manifold.*—With 10 outlets each connected to 50 mm long capillary tube, 1 mm bore. Cap unused outlets. (*See* Fig. 53, left.)

(5) *Nitrogen manometer.*—Construct from 6 mm o.d. tubing bent in shape of U-tube, height ca 380 mm, width ca 30 mm. Fill manometer ca half full with H_2O contg 1 drop Me orange and 1 drop H_2SO_4. To adjust flow of N, attach ca 3 ft rubber tubing to one of N outlets on distributing head. Fill 100 ml graduated cylinder with H_2O and invert in container of H_2O. Insert end of rubber tubing under cylinder. Turn on N supply and measure rate of displacement of H_2O in cylinder. Rate of flow should be 50–100 ml/min. Mark level of liquid in manometer at this flow rate.

(**b**) *Spectrophotometer.*—Covering spectral range of 220–360 mμ with wavelength scale readable to 0.1 mμ. Beckman Model DU is satisfactory. Adjust H lamp with no cell in beam so meter balances at lowest possible wavelength (usually 211 mμ or less). Slit widths are critical for absorption measurements at 262, 268, 274 mμ where, at final balancing, adjustments must be 0.8–0.9 mm.

(**c**) *Absorption cells.*—Quartz, matched pairs in lengths 1.000 ± 0.005 cm. When filled with H_2O or *iso*-octane, must match within 0.01 absorbance unit.

26.037 REAGENTS

(**a**) *Methanol, absolute.*—Check absorbance of 1 cm layer of MeOH against H_2O at 220 mμ and thru range of wavelengths used in analysis. Absorbance at 220 mμ must be <0.4 and curve should be smooth in range 262–322 mμ. Otherwise purify as follows, and recheck absorbance:

Place 2 L MeOH from new drum or glass bottles into 3 L double-neck ₸ distg flask; add 10 g KOH and 25 g Zn dust. Stopper one outlet and place reflux condenser in other, and reflux on steam bath 3 hr. Remove from steam bath; replace reflux tube with distg trap, 75° connecting tube, and condenser. Place flask in H_2O bath or elec. heating mantle and distill, collecting distillate in 2 L erlenmeyer. Store in g-s. bottle. EtOH is satisfactory if of comparable purity (as obtained or purified).

(**b**) *Iso-octane (2,2,4-trimethylpentane).*—NBS certified grade, Rohm and Haas Co., Philadelphia, Pa.; spectro grade, Phillips Petroleum Co., Bartlesville, Okla. Hexane or cyclohexane is satisfactory if absorbance requirements are met. Purify as follows: Place ca 3.5″ glass wool above stopcock at lower end of 32×1.75″ filter tube. Add ca 12″ silica gel (40–60 mesh, code 11-08, Davison Chemical Co., Baltimore, Md., or equiv.). Pour *iso*-octane slowly into tube, filling ca ¾ full. Insert cork stopper covered with Al foil loosely in

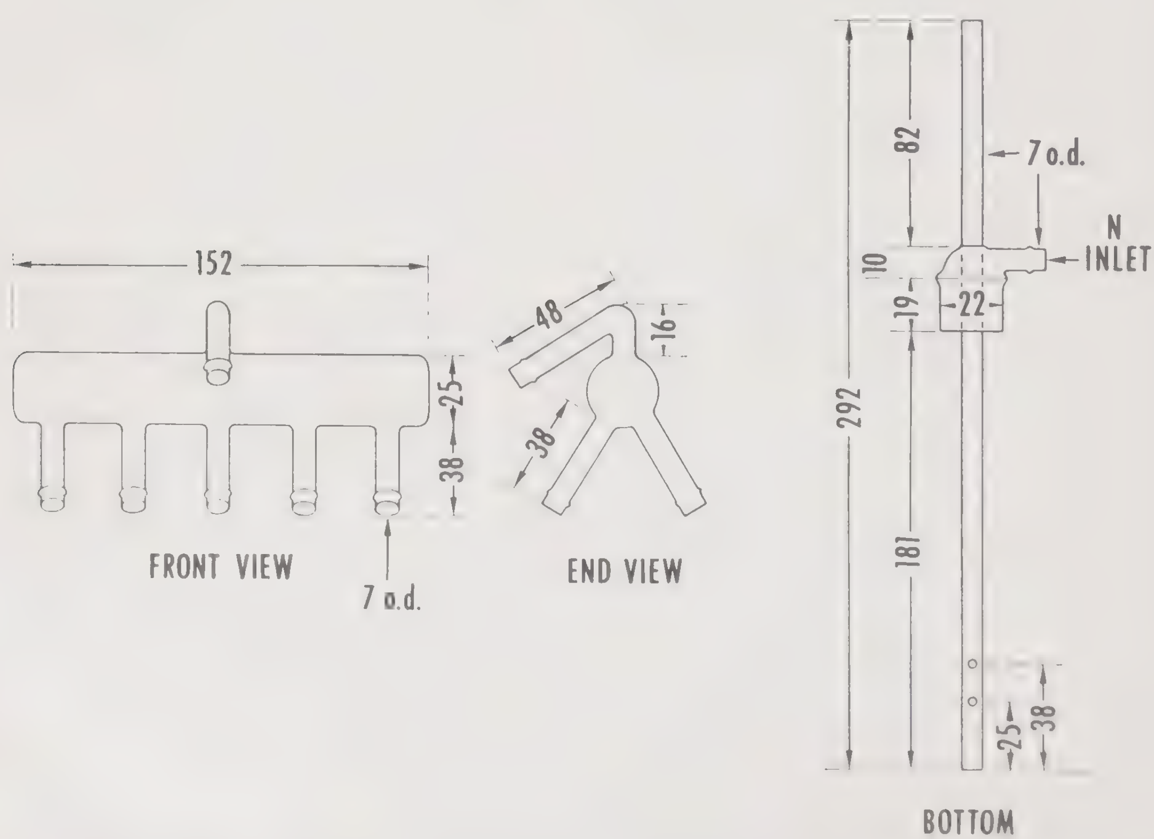


FIG. 53.—RIGHT, DISTRIBUTION HEADS. LEFT, MANIFOLD. ALL DIMENSIONS ARE IN MM

top of tube and let *iso*-octane filter thru silica gel, collecting in 2 L erlenmeyer. Renew silica gel as often as necessary to yield *iso*-octane conforming to absorbance limit. Check absorbance of 1 cm layer of the *iso*-octane against H_2O thru range of wavelengths used in analysis. Absorbance compared with H_2O set at 0 must be not >0.070 at all wavelengths and the resultant absorbance versus wavelength curve must be smooth. Otherwise refilter and recheck absorbance.

(**c**) *Potassium hydroxide-glycol soln.*—6.6% KOH for 25 min. isomerization. Weigh ca 750 g ethylene glycol into 1 L round-bottom Pyrex flask. Close with hollow stopper contg short outlet tube and inlet tube reaching to bottom of flask. Connect inlet tube to O-free N supply (<0.01% O) and bubble N thru liquid during all stages of prepn to exclude all air and to agitate liquid slightly. Place in oil bath at 100–150°; raise bath temp. to 190° and hold 10 min. to dry glycol. Remove bath and let bath temp. drop to 120°. Then slowly and carefully add 60 g 85% KOH, keeping soln under N. Return to oil bath; reheat bath to 190° and hold at this temp 10 min. Remove from bath, and cool. Remove hollow stopper and close with solid stopper. Store in refrigerator at ca 40°F under N.

Check KOH content by adding 10.00 g of the KOH-glycol soln to ca 90 ml MeOH, neutralized with 1*N* HCl to phthln end point. Titr. with stdzd 1*N* HCl until pink just disappears. % KOH = ml × normality × 5.61/wt soln. If % KOH is not 6.5–6.6, dry some glycol by heating under N at 190° as above, and adjust to 6.6% KOH.

(**d**) *Potassium hydroxide-glycol soln.*—21% KOH for 15 min. isomerization. Prep. as in (**c**) except use 210 g 85% KOH. Titr., and adjust to 21 ± 0.1% KOH, if necessary.

(**e**) *Nitrogen gas.*—Pre-purified grade, <0.01% O.

26.038 PREPARATION OF SAMPLE

Melt sample carefully on steam bath, stir thoroly, and filter if not clear.

26.039 DETERMINATION

(The 6.6% KOH method is preferred when samples contain only linoleic and linolenic acids; the 21% KOH method is preferred when samples contain linoleic, linolenic, and arachidonic acids. When pentaenoic acids are present, 21% KOH method must be used.)

(**a**) *For conjugated polyunsaturated acids.*—Weigh into 1 ml Pyrex cup (diam. 14 mm, height 10 mm) enough sample to give absorbance reading of 0.2 or more (ca 200 mg). Drop cup into 75 ml *iso*-octane in 150 ml beaker, and rotate beaker to dissolve sample, warming if necessary. Cool to room temp., transfer to 100 ml g-s. vol. flask, dil. to vol. with solvent, and mix thoroly. Measure absorbance in ultraviolet region against matched cell contg solvent, dilg soln (and/or using other cell lengths if necessary so that observed absorbance is 0.2–0.8). Also take readings on both sides of specified wavelengths to det. that max. is present. Component is considered absent if max. is not found in characteristic region and no further calcns are made in this region. Measure at: Dienoic, *233* mμ; trienoic, 262, *268*, 274 mμ; tetraenoic, 308, *315*, 322 mμ; pentaenoic, *346* mμ.

(**b**) *For non-conjugated polyunsaturated acids, 6.6% KOH, 25 min. isomerization.*—Weigh 100 mg (to nearest 0.5 mg) sample into 1 ml Pyrex glass cup. Weigh 11.0 ± 0.1 g of the 6.6% KOH-glycol soln into 10 × 1″ Pyrex test tube. Conduct at least 2 blank detns with sample. Cover tube with distributing head and connect to a capillary tube on manifold. Adjust flow of N to permit at least 50–100 ml N to pass thru tube/min. Let N sweep thru tube 1 min. to remove air; then immerse to depth of 4.5″ in bath at 180 ± 1°. Check temp. frequently and stdze thermometer at frequent intervals.

After 20 min. remove distributing head and drop 1 ml cup contg weighed sample into tube, note exact time, and replace head. Drop clean 1 ml cup into blanks. Keeping distributing head in place, remove tube from bath, swirl vigorously few sec., and return to bath. After 1 min. in bath, examine soln; if clear, return to bath. If not clear, indicating incomplete saponification, swirl tube 2–3 times and return to bath. At 1 min. intervals, repeat swirling until saponification is complete. Keep bath temp. at 180 ± 1°.

Exactly 25 min. after dropping sample into tube, remove from bath, wipe clean, and place in 3 L beaker to cool, continuing to pass N over soln. Cold H_2O bath may also be used. After cooling, remove head, and wash lower tubing with 20 ml purified MeOH, collecting washings in test tube. Wash with MeOH from beaker; do not use wash bottle.

Insert glass stirring rod 12″ long with curved end at bottom into test tube and move cup up and down to mix soln. Transfer soln to 100 ml g-s. vol. flask, dil. to vol. with purified MeOH, and mix thoroly. Measure absorbance as in (**a**), using KOH-glycol blank as reference. If diln of sample soln is required, make similar dilns of blank. If blanks do not check, repeat tests, increasing flow of N.

(**c**) *For non-conjugated polyunsaturated acids, 21% KOH, 15 min. isomerization.*—Proceed as in (**b**), except use 80 mg sample and 21 ± 0.1% KOH-glycol soln, and isomerize exactly 15 min.

26.040 SPECTROPHOTOMETRIC READINGS

If polyunsatd fatty acid constituent is known to be absent, or its absence is confirmed during

analysis (no max. detected at its analytical wavelength), no spectrophotometric reading is required in region of its absorption and no absorptivities at that region need be included in equations. For example, cottonseed oil is known to contain no polyunsatd constituents more highly unsatd than dienoic (linoleic acid). Hence in analysis of this oil, measurements are required only at 233 mμ, and equation to calc. linoleic acid content requires absorptivities only at this wavelength.

Correction for background absorption is used only when measuring very small traces of fatty acids. When fatty acid is present in more than trace, background corrections are not required and their use may lead to erroneous results. When absorptivity of any polyunsatd constituent after isomerization, at its analytical wavelength, is >1.0, no background correction should be made. No background corrections are to be made after isomerization with 21% KOH.

26.041 CALCULATIONS

(**a**) *Absorptivity for conjugated constituents.*—Calc. absorptivity, a, for each wavelength recorded in detn, **26.039**(a), using subscripts, 233, 268, 315, 346, to designate each individual a. $a = A/bc$, where A = observed absorbance, b = cell length in cm, and c = g sample/L final diln used for absorbance measurement.

In following equations, subscripts 2, 3, 4, and 5 refer to diene, triene, tetraene, and pentaene constituents, resp.

Absorptivity at 233 mμ corrected for absorption by acid or ester groups $= a_2 = a_{233} - a_0$ where $a_0 = 0.07$ for esters and 0.03 for soaps and fatty acids.

Absorptivity at 268 mμ corrected for background absorption $= a_3 = 2.8\ [a_{268} - \frac{1}{2}(a_{262} + a_{274})]$.

Absorptivity at 315 mμ corrected for background absorption $= a_4 = 2.5\ [a_{316} - \frac{1}{2}(a_{308} + a_{322})]$.

Absorptivity at 346 m$\mu = a_5 = a_{346}$.

(**b**) *Conjugated acids.*—If quantities within brackets of a_3 or a_4 are 0 or negative, no characteristic absorption maxima are present and corresponding constituent is reported as absent. As preformed constituents are usually present in small quantities, background absorption corrections are usually required. If large quantities of preformed constituents are present, this method is not applicable. However, no background corrections are to be applied to readings in pentaenoic region, 346 mμ.

% Conjugated diene $= C_2 = 0.91a_2$.

% Conjugated triene $= C_3 = 0.47a_3$.

% Conjugated tetraene $= C_4 = 0.45a_4$.

% Conjugated pentaene $= C_5 = 0.39a_5$.

(**c**) *Absorptivities for non-conjugated constituents, 6.6% KOH, 25 min. isomerization.*—Calc. absorptivity, a', for each wavelength in detn, (**b**). $a' = A/bc$.

Absorptivity at 233 mμ corrected for conjugated diene acids originally present $= a'_2 = a'_{233} - a_2 - 0.03$.

Absorptivity at 268 mμ corrected for background absorption and for undestroyed conjugated triene $= a'_3 = 4.03[a'_{268} - \frac{1}{2}(a'_{262} + a'_{274})] - a_3$.

Absorptivity at 315 mμ corrected for background absorption and for undestroyed conjugated tetraene $= a'_4 = 2.06[a'_{315} - \frac{1}{2}(a'_{308} + a'_{322})] - a_4$.

(**d**) *Non-conjugated acids, 6.6% KOH, 25 min. isomerization.*—(1) Without background corrections:

% Linoleic acid $= X = 1.086a'_2 - 1.324(a'_{268} - a_{268}) + 0.40(a'_{315} - a_{315})$.

% Linolenic acid $= Y = 1.980(a'_{268} - a_{268}) - 4.92(a'_{315} - a_{315})$.

% Arachidonic acid $= Z = 4.69(a'_{315} - a_{315})$.

(2) When background corrections are required:

% Linoleic acid $= X = 1.086a'_2 - 1.324a'_3 + 0.40a'_4$.

% Linolenic acid $= Y = 1.980a'_3 - 4.92a'_4$.

% Arachidonic acid $= Z = 4.69a'_4$.

(**e**) *Absorptivities for non-conjugated constituents, 21% KOH, 15 min. isomerization.*—Calc. absorptivities a' for each wavelength 233, 268, 315, and 346 mμ. (If no max. is found, report component as 0 without further measurement or calcn.)

Absorptivity at 233 m$\mu = a'_2 = a'_{233} - a_2$.

Absorptivity at 268 m$\mu = a'_3 = a'_{268} - a_{268}$.

Absorptivity at 315 m$\mu = a'_4 = a'_{315} - a_{315}$.

Absorptivity at 346 m$\mu = a'_5 = a'_{346} - a_{346}$.

(**f**) *Non-conjugated acids, 21% KOH, 15 min. isomerization.*—(Spectrophotometric method will not differentiate between acids with same number of double bonds but different chain length, e.g., between C_{20} and C_{22} pentaenes. First 2 sets of equations below are for samples contg C_{20} pentaene acid and for samples contg C_{22} pentaene acid, resp. If chain length is unknown, assume that these pentaene acids are present in equal quantities, and apply third set of equations.)

(1) *Samples contg C_{20} pentaene acid:*

% Linoleic acid $= X = 1.09a'_2 - 0.57a'_3 - 0.26a'_4 + 0.002a'_5$.

% Linolenic acid $= Y = 1.10a'_3 - 0.88a'_4 + 0.31a'_5$.

% Arachidonic acid $= Z = 1.65a'_4 - 1.55a'_5$.

% Pentaenoic acids $= P = 1.14a'_5$.

(2) *Samples contg C_{22} pentaene acid:*

% Linoleic acid $= X = 1.09\,a'_2 - 0.57\,a'_3 - 0.26a'_4 - 0.12a'_5$.

% Linolenic acid $= Y = 1.10a'_3 - 0.88a'_4 - 0.02a'_5$.

% Arachidonic acid $= Z = 1.65a'_4 - 1.86a'_5$.

% Pentaenoic acids $= P = 1.98a'_5$.

(3) *Samples contg pentaene acids of unknown chain length (calcd as 50% C_{20}–50% C_{22} pentaenoic acids)*:

% Linoleic acid $= X = 1.09a'_2 - 0.57a'_3 - 0.26a'_4 - 0.03a'_5$.

% Linolenic acid $= Y = 1.10a'_3 - 0.88a'_4 + 0.19a'_5$.

% Arachidonic acid $= Z = 1.65a'_4 - 1.67a'_5$.

% Pentaenoic acids $= P = 1.45a'_5$.

(g) *Total composition:*

% Total conjugated polyunsaturated acids $= C_2 + C_3 + C_4 + C_5$.

% Total nonconjugated polyunsatd acids $= X + Y + Z + P$.

% Oleic acid $= \{$I value (Wijs) of sample $- [1.811(C_2 + X) + 2.737(C_3 + Y) + 3.337(C_4 + Z) + 4.014^*(C_5 + P)]\}/0.899$.

% Satd acids = % total fatty acid − (% oleic acid + % conjugated acid + % nonconjugated acid).

(% total fatty acid of most naturally occurring oils is 95.6. To calc. to fatty acid basis, multiply the % value by 100/% total fatty acid.)

Free Fatty Acids in Crude and Refined Oils

26.042 *National Cottonseed Products Association Method—Official*

(a) *In crude oils.*—Weigh 7.05 g well-mixed oil into 250 ml flask or 4 oz bottle. Add 50 ml alcohol, previously neutralized by adding 2 ml phthln soln and enough 0.1*N* NaOH, **42.032–42.033,** to produce faint permanent pink. Titr. with 0.25*N* NaOH with vigorous shaking until permanent faint pink appears and persists at least 1 min. Report as % free fatty acids expressed as oleic acid. Ml 0.25*N* NaOH used in titrn corresponds to this percentage.

(b) *In refined oils.*—Put ca 50 ml alcohol into clean, dry 150 ml flask, and add few drops of the oil and 2 ml phthln. Place flask in H_2O at 60–65° until warm, and add enough 0.1*N* NaOH to produce faint permanent pink. Weigh 56.4 of the oil into the neutralized alcohol and titr., occasionally warming and violently shaking mixt. until same faint permanent pink appears in supernatant alcohol. Multiply ml 0.1*N* NaOH by 0.05 and report as % free fatty acids expressed as oleic acid.

Acetyl Value (*14*)—Official

26.043 ACETYLATION

Boil 50 ml sample 2 hr with 50 ml freshly distd Ac_2O under reflux condenser. Pour mixt. into 500 ml H_2O in beaker and boil 15 min., while bubbling stream of air or CO_2 thru soln to prevent bumping. Siphon off H_2O, add 500 ml more H_2O, and boil again 15 min. Repeat siphoning and boil 15 min. with third 500 ml portion H_2O. Let mixt. cool and sep. aq. layer, which should be neutral to litmus. Transfer acetylated oil to separator and wash with two 200 ml portions warm H_2O. Sep. as much of the H_2O as possible, add 5 g anhyd. Na_2SO_4 to acetylated oil, and let stand 1 hr, agitating occasionally to assist drying. Filter thru dry folded filter, preferably in oven at 100–110°, and keep filtered oil in oven until completely dry. Acetylated product should be clear, brilliant oil.

26.044 SAPONIFICATION

Weigh accurately 2–2.5 g each of acetylated oil and untreated oil into sep. 250 ml erlenmeyers. Add to each flask exactly 25 ml alc. KOH soln, **26.022,** and reflux 1 hr. Titr. warm solns with 0.5*N* HCl, using phthln. Titr. two 25 ml portions of the alc. KOH soln in same way. From av. of these 2 results, which should be in very close agreement, deduct vol. of the std HCl required for titrn of acetylated and of untreated oil, and from results so obtained calc. saponification number (mg KOH required to saponify 1 g product) of each. Calc. acetyl value by following formula:

$A = (S' - S)/(1 - 0.00075S)$, where A = acetyl value; S = saponification number of oil; and S' = saponification number of acetylated oil.

Cholesterol and Phytosterol in Mixtures of Animal and Vegetable Fats

Sterol Acetate Method (15)—First Action

26.045 APPARATUS

(a) *Special micro filter.*—*See* Fig. 54.

(b) *Platinum spatula.*—Heavy Pt wire, hammered flat, to ca 3 mm wide × 15 mm long, on one end. Mount in dissecting needle holder.

26.046 DETERMINATION

To 15 g filtered fat in 150 ml beaker add 4 g KOH dissolved in 4 ml H_2O. Add 20 ml 95% alcohol, cover with watch glass, and heat 0.5 hr on steam bath, stirring occasionally.

Add 60 ml H_2O, mix, and pour into 400 ml beaker contg 180 ml 95% alcohol. Warm to ca 40° and add 40 ml *1% digitonin in alcohol.* (Heat may be necessary to dissolve digitonin.) Stir and let stand overnight in refrigerator.

Filter cold mixt. with strong suction on rapid qual. 11 cm paper in büchner. When liquid has passed thru paper, pour 50 ml H_2O over paper without stopping suction. Swirl occasionally. Continue to apply strong suction (H_2O passes thru paper rather slowly) until all H_2O has passed thru paper to wash out most of soaps. Pour 50 ml alcohol over paper and continue suction until

* Corresponding constant for sample contg all C_{20} pentaene acids is 4.197; for all C_{22} pentaene acids is 3.841.

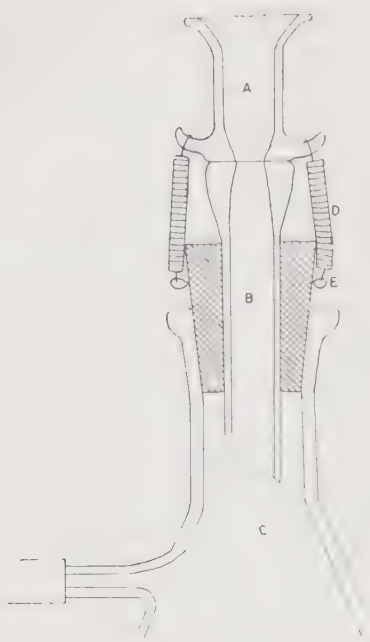


FIG. 54.—GLASS MICRO FILTER FOR STEROL ACETATE PRECIPITATES

A: Top portion of filter, capacity 1 ml. **B:** Lower portion of filter. Ground surfaces between **A** and **B** hold filter pad. **A** and **B** are held together by springs **D. C,** filter flask. **E,** wire twisted around stopper to hold lower end of springs.

all liquid has passed thru. Finally wash paper with four 50 ml portions ether, letting each portion pass thru completely before adding next portion.

Dry paper and ppt 15 min. at 100°. Sep. ppt from paper. Crush or crumble ppt, and place it in 18×150 mm test tube. Add 2 ml Ac_2O and heat in 130° glycerol bath 15 min. (Ppt should dissolve in ca 5 min.; do not use direct heat, since spattering may occur and material may be lost.)

Carefully add 4 ml alcohol and mix. Filter hot soln by gravity thru pledget of cotton in micro filtering tube (Pregl type), receiving filtrate in 20 ml beaker. Place beaker on small hot plate and carefully bring liquid to gentle boil. Add H_2O drop by drop until sterol acetate is just about to ppt but still remains in soln at b.p.

Let cool, stirring occasionally with the Pt spatula, for 15–20 min. or longer. Filter on small disk of paper in micro büchner (ca 15 mm diam.). Suck dry and sep. ppt from paper. Place ppt in 5 ml beaker and heat with 1 ml 95% alcohol to dissolve completely. Cool beaker by setting in petri dish of ice-H_2O. When thoroly chilled, material usually sets to semisolid cryst. slurry.

Transfer slurry to special micro-filter, using Pt spatula, and apply suction. As liquid is drawn thru filter, compact ppt by tamping with flat end of glass rod of suitable size. (Ppt can then be cleanly and completely removed from paper in form of small button or tablet.) Redissolve ppt in same 5 ml beaker with addnl 1 ml hot alcohol (or 0.5 ml if ppt is very small), and after chilling to recrystallize, filter second time on micro-filter. Repeat recrystn and filtration third and fourth time; then dry ppt 1 hr at 100°.

Det. m.p. of recrystd, dried sterol acetates (temp. at which liquid first starts to run, detd when heated at rate of 0.5–1.0°/min.). If m.p. is 2° or more higher than that of pure butter similarly treated, vegetable fat is indicated.

26.047 MICROCRYSTAL TEST

Dissolve sterol acetate remaining from m.p. detn in 2 ml alcohol in 20 ml beaker and add 3 drops 40% aq. KOH. Heat on steam bath 5 min. Add 10 ml H_2O and transfer liquid to 125 ml separator. Add 25 ml ether and shake. Let layers sep.; then drain and discard aq. layer. Wash ether with three 5 ml portions H_2O and evap. ether to dryness in 50 ml beaker.

Add 10 ml 70% alcohol to residue and heat to dissolve. Cool and place drop of clear soln on slide, and examine drop microscopically at 100–200× for typical crystals of phytosterol or phytosterol-cholesterol mixt. (*see* Fig. 55).

26.048 Digitonin Recovery—Procedure

Combine filtrates from digitonide pptns and add enough *cholesterol* dissolved in alcohol to combine with all the digitonin present. Let mixt. stand 3 hr or overnight. Filter off ppt and wash with H_2O, alcohol, and ether; then suck dry. Crush ppt and tamp it lightly into paper extn thimble. Suspend thimble in ₸ erlenmeyer closed by reflux condenser and contg small amount of *xylol.* Heat xylol to boiling and let thimble and contents hang in hot vapors of boiling xylol 16 hr.

Remove thimble and dry at 100° until xylol has evapd. Remove digitonin residue, weigh, and transfer to beaker. Dissolve residue in enough H_2O to make ca 2% digitonin soln. Add ca ½ vol. alcohol and heat on steam bath. Add 1 ml *n-amyl alcohol* (reagent grade), cool, and filter off digitonin compound on büchner of suitable size. Suck dry and transfer ppt to watch glass. Dry at 100° until all amyl alcohol is volatilized. Digitonin may then be pulverized and is ready for re-use.

26.049 Unsaponifiable Residue (*16*)—Official

Weigh accurately 2–2.5 g fat into saponification flask (200 ml erlenmeyer with ₸ 24/40 outer joint is recommended). Add 25 ml alcohol and 1.5 ml KOH soln (3+2). Saponify by boiling, with occasional swirling, on steam bath 30 min. under reflux air condenser. (No loss of alcohol should

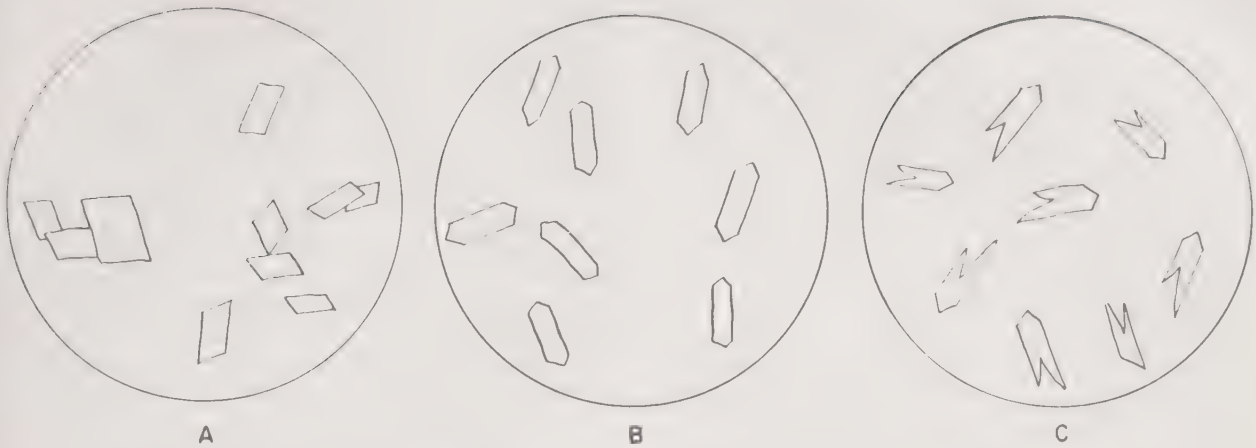


FIG. 55. CRYSTALLINE FORMS OF STEROL ACETATES. A: CHOLESTEROL. B: PHYTOSTEROL. C: MIXED CHOLESTEROL-PHYTOSTEROL.

occur during saponification.) Transfer alc. soap soln while still warm to 250 ml separator, using total of 50 ml H_2O. Rinse saponification flask with 50 ml ether and add ether to separator. Shake vigorously and let layers sep. and clarify. Drain lower layer and pour ether layer thru top into second separator contg 20 ml H_2O. Rinse pouring edge with ether, adding rinsings to second separator. Make 2 more extns of the soap soln with 50 ml portions ether in same manner. Make total of 4 extns in case of marine oils or other oils of high unsaponifiable content.

Rotate combined ether exts gently with the 20 ml H_2O (violent shaking at this stage may cause troublesome emulsions). Let layers sep. and drain aq. layer. Wash with two addnl 20 ml portions H_2O, shaking vigorously. Then wash ether soln 3 times with alternate 20 ml portions ca 0.5*N* aq. KOH and H_2O, shaking vigorously each time. If emulsion forms during washing, drain as much of aq. layer as possible, leaving emulsion in separator with ether layer, and proceed with next washing. After third KOH treatment, wash ether soln successively with 20 ml portions H_2O until washings are no longer alk. to phthln.

Transfer ether soln to 250 ml lipped, conical beaker, rinse separator and its pouring edge with ether, and add rinsings to main soln. Evap. to ca 5 ml and transfer quantitatively, using several small portions of ether, to 50 ml fat flask or erlenmeyer previously dried and weighed with similar flask as tare. Evap. ether. When nearly all ether has been removed, add 2–3 ml acetone, and while heating on steam or H_2O bath, completely remove solvent in gentle current of air. Dry at 100° for 30 min. periods to constant wt.

Dissolve contents of flask in 2 ml ether, add 10 ml neutralized (phthln) alcohol and titr. with 0.1*N* alc. NaOH (or KOH). (Not >0.10 ml is usually required.) Correct wt residue for free fatty acid present (1 ml 0.1*N* alkali = 0.0282 g oleic acid).

Correct wt residue for reagent blank obtained by conducting detn in same manner but omitting fat.

Squalene (*17*)—Official

26.050 REAGENTS

(**a**) *Concentrated potassium hydroxide soln.*—Dissolve 60 g KOH in 40 ml H_2O.

(**b**) *Dilute potassium hydroxide soln.*—Dissolve 28 g KOH in H_2O and dil. to 1 L.

(**c**) *Petroleum ether.*—Skellysolve B (b.p. 63-70°) or equiv.

(**d**) *Aluminum oxide adsorbent, 80–200 mesh.*—Adsorption alumina for chromatographic analysis. Keep in tightly closed container, away from moisture.

(**e**) *Pyridine sulfate bromide soln.*—0.1*N*. Dissolve 8 g Br in 20 ml HOAc (99.5%). Prep. another soln by adding gradually, with cooling, 5.45 ml H_2SO_4 to mixt. of 20 ml HOAc and 8.15 ml pyridine. Mix 2 solns, cool, and dil. to 1 L with HOAc.

(**f**) *Sodium thiosulfate soln.*—0.05*N*. Dissolve 13 g $Na_2S_2O_3.5H_2O$ in CO_2-free H_2O contg 1% *iso*-amyl alcohol, dil. to 1 L, mix, and filter. Stdze against exactly 0.05*N* KIO_3 (1.7835 g/L) as follows: To g-s. 125 ml erlenmeyer, add 10 ml 10% KI soln, 5 ml H_2O, 2 g $NaHCO_3$, and, slowly, 5 ml ca 6*N* HCl. Mix, add 25 ml of the KIO_3 soln, wash down sides of flask with H_2O, and titr. at once with the $Na_2S_2O_3$ soln, using starch indicator, **2.093**(**d**), toward end of titrn.

26.051 APPARATUS

Adsorption column.—Prep. fresh column for each detn immediately before use. Place small

wad of cotton in constricted end of glass tube, 8 mm i.d. and 30 cm long. Add Al_2O_3 adsorbent in ca 10 small portions until column is ca 10 cm high. Apply gentle suction and tamp each portion of the Al_2O_3 lightly with flattened end of heavy glass rod. Place small wad of cotton on top of column and tamp lightly. Wash column with ca 15 ml petr. ether, remove suction, and keep top of column covered with shallow layer of petr. ether until ready for use.

26.052 DETERMINATION

Weigh accurately (± 20 mg) ca 5 g sample into 125 ml erlenmeyer with ₸ joint, add 3 ml of the concd KOH soln and 20 ml alcohol, and boil mixt. under air condenser 30 min., shaking occasionally. Cool somewhat, and while still warm, add 50 ml petr. ether; mix, and transfer to separator. Rinse flask with 20 ml alcohol and then with 40 ml H_2O, adding rinsings to soln in separator. Shake vigorously, let sep. completely, and slowly drain soap soln. Pour petr. ether ext. from top of separator into another separator contg 20 ml H_2O. Repeat extn of soap soln with 50 ml petr. ether. Rotate combined exts gently with the 20 ml H_2O and, after letting layers sep., discard wash H_2O. Repeat washing by shaking vigorously with 20 ml H_2O and again discard lower layer after sepn. Wash petr. ether soln with 20 ml of the dil. KOH soln and then with successive 20 ml portions H_2O until wash liquid is alkali-free, shaking vigorously each time. After final washing, drain last drops of H_2O brought down by swirling separator. Pour petr. ether soln from top of separator into lipped conical beaker. Rinse separator with petr. ether and add rinsings to beaker. Add few pieces of broken porcelain or SiC and evap. almost all of solvent on steam bath. Remove last traces of solvent in current of CO_2 or other inert gas while warming beaker. To avoid oxidation of residue, do not expose to air while still warm.

Dissolve unsaponifiable matter in 5 ml petr. ether and transfer to adsorption tube. (Filtrate, which is caught in 250 ml g-s. I flask, should emerge dropwise, ca 1 ml/min., gentle suction being used if necessary.) When soln has been nearly drawn into column, add ca 5 ml petr. ether previously used to rinse beaker. Continue adding solvent in 5–10 ml portions previously used to rinse beaker, always keeping surface of column covered with liquid, until total vol. of 50 ml has passed thru adsorption tube. Add few pieces of broken porcelain or SiC and remove most of solvent on steam bath. Finally pass current of CO_2 or other inert gas thru heated flask until last traces of solvent are expelled. Cool residue to room temp. under inert atmosphere. (All traces of solvent must be removed before detn is continued.)

Dissolve unadsorbed residue in 5 ml $CHCl_3$ and add enough of the pyridine sulfate bromide soln to provide at least 50% excess (10 ml is usually adequate). Let mixt. remain in dark 5 min. and then add 5 ml 10% KI soln, together with 40 ml H_2O. Mix thoroly, wash down any free I on stopper, and titr. with the $0.05N$ $Na_2S_2O_3$. Toward end of titrn add starch indicator, **2.093(d)**, shake flask vigorously, and continue titrn to disappearance of blue color. Conduct blank detn on the pyridine sulfate bromide soln in same manner and calc. ml $0.05N$ $Na_2S_2O_3$ equiv. to absorbed halogen. Blank detn on all reagents used should show practically no halogen consumption. 1 ml $0.05N$ $Na_2S_2O_3 = 1.71$ mg squalene. Report results as mg squalene/100 g sample.

Rosin Oil

26.053 *Qualitative Test—Procedure*

Polarize pure oil, or definite diln with petr. ether, in 200 mm tube. Rosin oil has polarization in 200 mm tube of +30–40° S, while most oils (*18*) read between +1° and −1°.

Cottonseed Oil

26.054 *Halphen Test (19)—Official*

Mix CS_2 contg 1% S in soln with equal vol. amyl alcohol. Mix equal vols of this reagent and sample under examination, and heat in bath of boiling satd NaCl soln 1–2 hr. Presence of as little as 1% cottonseed oil produces pronounced characteristic red or orange-red color. Depth of color is proportional, to certain extent, to quantity of cottonseed oil present, and comparative tests with known mixts of cottonseed oil give approximation of quantity.

Different oils react with different intensities. Oils that have been heated to 200–210° (*20*) and hydrogenated oils (*21*) react with greatly diminished intensity. Heating 10 min. at 250° renders cottonseed oil incapable of giving reaction (*22*). Fat of animals fed on cottonseed meal or other cottonseed products may give positive reaction by this test.

Peanut Oil

26.055 *Modified Renard Test (23)—Official*

Weigh 20 g oil into erlenmeyer. Saponify with alc. KOH soln, **26.022**; neutralize exactly with HOAc (1+3), using phthln; and wash into 800–1000 ml flask contg boiling mixt. of 100 ml H_2O and 120 ml 20% $Pb(OAc)_2$ soln. Boil 1 min. and then cool pptd soap by immersing flask in H_2O, swirling flask occasionally to cause soap to stick to sides. After flask cools, decant the H_2O and excess $Pb(OAc)_2$ soln, and wash the Pb soap with

cold H_2O and alcohol, 90% by vol. Add 200 ml ether, cork, and let stand until soap disintegrates; heat on H_2O bath, using reflux condenser, and boil ca 5 min. (*24*). With oils, most of soap will be dissolved, while with lards, which contain much stearin, part of soap will be left undissolved. Cool ether soln of soap to 15–17° and let stand until all insol. soaps sep. (ca 12 hr).

Filter on büchner and thoroly wash insol. Pb soaps with ether. Wash ether-insol. Pb soaps into separator with jet of ether, alternating with HCl (1+3) at end of operation if little of soap sticks to paper. Add enough HCl (1+3) so that total vol. of acid is ca 200 ml and enough ether to make its total vol. 150–200 ml, and shake vigorously several min. Let layers sep., drain off acid layer, and wash ether once with 100 ml HCl (1+3) and then with several portions of H_2O until H_2O washings are no longer acid to Me orange. If few undecomposed lumps of Pb soap remain (indicated by solid particles remaining after third washing with H_2O), break up by running off almost all H_2O layer, adding little HCl, and shaking; then continue washing with H_2O as before.

Distill ether from soln of insol. fatty acids and dry latter in flask by adding little absolute alcohol and evapg on steam bath. Dissolve dry fatty acids by warming with 100 ml 90% alcohol by vol. Cool slowly to 15°, shaking to aid crystn. Let stand 30 min. at 15°.

In presence of peanut oil, crystals of arachidic acid sep. from soln. Filter, wash ppt twice with 10 ml alcohol, 90% by vol., and then with alcohol, 70% by vol., taking care to keep arachidic acid and wash solns at definite temp. in order to apply solubility corrections given below. Dissolve arachidic acid on filter with boiling absolute alcohol, evap. to dryness in weighed dish, dry, and weigh. Add to wt 0.0025 g/10 ml of 90% alcohol used in crystn and washing, if conducted at 15°; if conducted at 20°, add 0.0045 g/10 ml.

M.p. of arachidic acid thus obtained is 71–72°. 20 times wt arachidic acid gives approx. quantity peanut oil present. Arachidic acid has characteristic appearance and may be identified under microscope. As little as 5–10% peanut oil can be detected by this method.

Modified Bellier Test (25)—Official

(Applicable only in presence of olive, cottonseed, corn, and soybean oils)

26.056 REAGENTS

(a) *Alcoholic potassium hydroxide soln.*—1.5*N*. Dissolve 10 g KOH in purified alcohol, **26.022**, and dil. to 100 ml with purified alcohol.

(b) *Hydrochloric acid.*—Sp. gr. 1.16. Dil. 83 ml concd acid (sp. gr. 1.19) to 100 ml with H_2O. Check with sp. gr. spindle.

(c) *Alcohol.*—70%. Dil. 700 ml alcohol to 950 ml with H_2O. Check by sp. gr. or refractive index and adjust if necessary.

26.057 TEST

Weigh 0.92 g or measure 1 ml sample into 125 ml erlenmeyer with ₸ outer joint. If oil is measured, use short Mohr pipet with fairly large opening at tip, drain to lower mark, hold until meniscus stops rising in pipet, and drain to mark again. Add 5 ml of the alc. KOH soln, and heat 5 min. on steam bath, using air condenser to avoid loss of alcohol. Swirl once or twice during saponification. Add 50 ml 70% alcohol and 0.8 ml of the HCl. Warm to dissolve any ppt that may form.

Insert thermometer and cool with continuous agitation so that temp. falls ca 1°/min. Observe turbidity temp. or clouding point, which is temp. at which definite ppt first appears. (If temp. of soln is above room temp., cooling may be accomplished in air or by occasionally immersing soln in H_2O bath of temp. not >5° below that of soln. Do not immerse flask below level of contents, and agitate continuously to prevent premature formation of turbidity by local cooling. Soln may be agitated by stirring with thermometer or by swirling flask. Observe turbidity temp. by looking thru soln toward good light, or toward dark background with good light coming from one side.)

If turbidity appears before temp. reaches 9° (olive oil) or 13° (cottonseed, corn, or soybean oils) presence of peanut oil is indicated. Confirm by **26.055**.

26.058 Cold Test (*26*)—Procedure

(Applicable to refined winterized salad oils)

Fill 4 oz oil sample bottle with the oil at 25°, cork tightly, and seal with paraffin. Submerge bottle completely in bucket contg finely cracked ice and add H_2O until it rises to top of bottle. Keep bucket filled solidly with the ice by removing any excess H_2O and adding ice when necessary. After 5.5 hr remove bottle and examine oil. If it is properly wintered, sample will be brilliant, clear, and limpid.

26.059 Tea Seed Oil in Olive Oil (*27*)—Official

For preliminary qual. test use following room temp. method: Measure into test tube (18×150 mm is convenient) exactly 0.8 ml Ac_2O, 1.5 ml $CHCl_3$, and 0.2 ml H_2SO_4. Mix, and cool to room temp. Add 7 drops of oil to be tested directly to reagents, mix, and cool again. (To measure test oil use glass tubing, 4 mm o. d., and ca 2 mm i. d.; 7 drops should weigh ca 0.22 g.) If soln of oil in reagents is cloudy after mixing and cooling, add

Ac_2O dropwise, shaking after each addn until soln suddenly clears. Appreciable deviations from these quantities, particularly in the H_2SO_4, cause distinct variations in color intensities. Since mixed reagent deteriorates slowly, do not mix in advance of testing.

After 5 min., add 10 ml absolute ether from cylinder and mix immediately by inverting once. Tea seed oil shows brown color changing to intense red within min. or so. This red color reaches max. and then fades slowly within few min. Olive oil forms initial green color on addn of ether. This color fades slowly to brown-gray, occasionally passing thru faint pink stage. Both olive oil and tea seed oil eventually fade to permanent light brown color. Mixts of tea seed oil and olive oil show characteristic tea seed oil colors proportional in intensity to quantity of tea seed oil present.

For approx. quant. estimations drop oil into reagents as described above and let remain at room temp. 5 min. In meantime, cool 10 ml portion absolute ether in ice-H_2O. After 5 min., place test tube contg oil and reagents in the ice-H_2O 1 min., add the cold ether (taking care that no H_2O falls into test tube), and mix. Return tube to ice-H_2O bath and let colors develop while it is immersed in the ice-H_2O. Colors develop slowly and reach max. within ca 5 min.

Use deepest red colors produced as basis for comparison, and because of short period of stable max. intensity do not test more than 3 oils at one time. Stds contg known quantities of tea seed oil in olive oil that give little or no pink color with this test should be run simultaneously with sample. Preliminary room temp. test gives indication of stds to be used in ice-H_2O method.

Sesame Oil

26.060 *Modified Villavecchia Test (28)—Official*

Add 2 ml furfural to 100 ml alcohol. Mix thoroly 0.1 ml of this soln with 10 ml HCl and 10 ml sample by shaking in test tube 15 sec. Let mixt. stand 10 min., observe color, add 10 ml H_2O, shake, and again observe color. If crimson color disappears, sesame oil is absent. (As furfural gives violet tint with HCl, it is necessary to use the very dil. soln specified.)

26.061 Foreign Fats Containing Tristearin in Lard (*29*)—First Action

Weigh 5 g of the melted and filtered lard into g-s. cylinder and add 20 ml warm acetone. Mix well, taking care that soln is clear and has temp. $>30°$. Let stand 16–18 hr at constant temp of 30°. Fine mass of crystals occupying not >3 ml should then be found at bottom of cylinder. Should vol. of crystals materially exceed 3 ml, take smaller quantity of lard (3–4 g) for new test. Should no crystals be deposited, as may be case with soft or oily lard, tristearin is probably absent.

Decant supernatant acetone soln from crystd glycerides. Add three 5 ml portions warm (30–35°) acetone from small wash bottle, taking care not to break up deposit in washing, and decant first 2 portions. Actively agitate third portion in cylinder and by quick movement transfer crystals to small filter paper. Using wash bottle, wash crystals with 5 successive small portions of the warm acetone and remove excess acetone by suction. Spread out paper and its contents, breaking up any large lumps, and dry in air at room temp.

Thoroly comminute mass and take m.p. of crystals in closed 1 mm tube, using app. similar to that of Fig. 56. Heat H_2O in beaker rapidly to ca 55° and maintain this temp. until thermometer carrying m.p. tube registers 50°; then heat again and raise temp. of outer bath rather quickly to

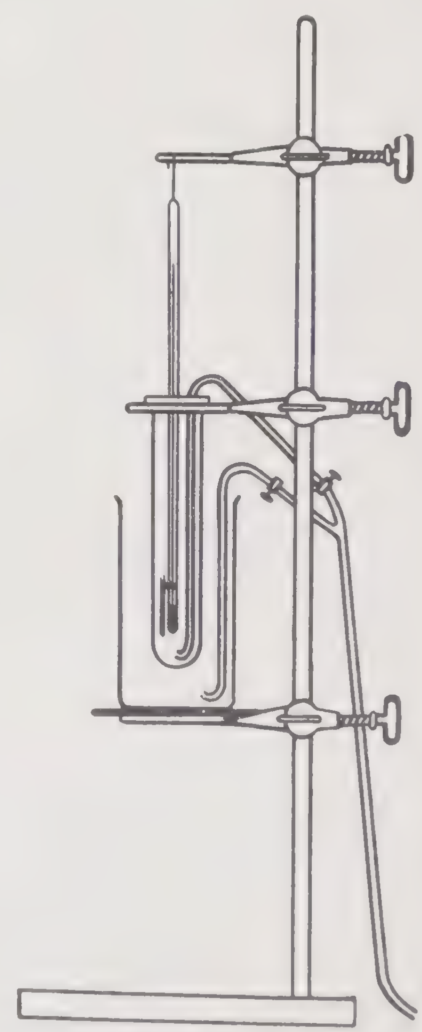


FIG. 56.—APPARATUS FOR DETERMINATION OF MELTING POINT

67°. Remove burner. M.p. is reached when fused substance becomes perfectly clear and transparent.

When m.p. of glycerides obtained by this method is <63.6°, presence of beef fat or other fat contg tristearin should be suspected, and m.p. of 63.2° or lower is evidence that sample is not pure lard. Conduct detn with control sample of pure lard.

Conclusion indicated by m.p. may be confirmed by taking m.p. of fatty acids prepd from glycerides. After detg m.p., transfer crystd glycerides to 50 ml beaker, add 25 ml ca 0.5N alc. KOH, and heat on steam bath until saponification is complete. Pour soln into separator contg 200 ml H_2O, acidify, add 75 ml ether, shake, and let stand. Drain aq. acid layer and wash ether soln at least 3 times with H_2O. Transfer ether soln to clean dry 50 ml beaker, evap. ether on steam bath, and finally dry acids at 100°. Let acids remain at room temp. ca 2 hr and det. m.p. If m.p. of glycerides, plus twice difference between m.p. of glycerides and m.p. of fatty acids, is <73°, the lard is regarded as adulterated.

Conclusions may be confirmed further by precise detns of mean molecular wt of sepd fatty acids. Dissolve acids in colorless, redistd alcohol neutralized carefully immediately before use, and titr. with 0.5–0.2N KOH, using phthln. Mean molecular wt = wt fatty acids × 1000/ml × normality KOH used. If sample is pure lard, mean molecular wt of fatty acids should correspond closely to that of fatty acids of α-palmito-distearin, 275.13. If sample is impure, mean molecular wt should approach that of fatty acids from tristearin, 284.49.

Fish Oil and Marine Animal Oils in Presence of Vegetable Oils and in Absence of Metallic Salts

26.062 *Qualitative Test—Procedure*

Dissolve in test tube ca 6 g sample in 12 ml mixt. of equal parts $CHCl_3$ and HOAc. Add Br, dropwise, until slight excess is indicated by color, keeping soln at ca 20°. Let mixt. stand 15 min. or more and place test tube in boiling H_2O. If vegetable oils only are present, soln is perfectly clear, but fish oils remain cloudy due to presence of insol. bromides.

Mineral Oil in Fats (*30*)

26.063 *Qualitative Test—Procedure*

Place 1 ml oil or melted fat in erlenmeyer; add 1 ml KOH soln (3+2) and 25 ml alcohol. Boil under reflux air condenser, shaking occasionally, until saponification is complete (ca 5 min.). Add 25 ml H_2O and mix. In presence of >0.5% mineral oil, distinct turbidity appears.

26.064 *Quantitative Method—Official*

Treat unsaponifiable matter, **26.049**, with H_2SO_4 as below. When very small quantities of mineral oil are present, enough unsaponifiable matter for test may be obtained as follows:

Saponify 100 g fat by refluxing under air condenser 2 hr with 55 ml KOH soln (3+2) and 240 ml alcohol, shaking flask occasionally. Cool, add 300 ml petr. ether (b.p. 35–60°), and transfer to separator. Rinse flask with 240 ml alcohol and add rinsings to separator. Add 480 ml H_2O and shake vigorously. Let layers sep., drain lower layer, and transfer upper layer to another separator. Repeat extn of saponified fat with 300 ml petr. ether and combine exts. Wash ext. twice with 60 ml portions H_2O, using gentle agitation. Repeat washing with 60 ml 0.5N KOH, followed by vigorous agitation with successive 60 ml portions H_2O until washings are alkali-free. Evap. ext. to small vol. and dry with anhyd. Na_2SO_4.

Filter petr. ether soln thru small cotton plug into Babcock milk-test bottle, **15.030(a)**, add few small pieces of broken porcelain, and remove solvent by heating on steam bath while passing current of air thru bottle. Cool, add 5 ml H_2SO_4, mix, and keep bottle in boiling H_2O bath 30 min., shaking occasionally. Remove bottle from bath, cool, and fill with H_2SO_4 until surface rises well into graduated neck. Centrifuge 5 min. at 1200 rpm and read vol. of unreacted residue. If enough mineral oil is available, obtain density as in **26.004**, using small Sprengel tube. Wt mineral oil can be closely approximated by multiplying vol. by 0.88. Refractive index of colorless residue should be <1.500 at 20°.

Coal-Tar Colors (*31*)—Official

26.065 REAGENTS

(**a**) *Acid soln A.*—Mix 1 L HOAc with 200 ml HCl and 100 ml H_2O.

(**b**) *Acid soln B.*—Cautiously add 400 ml H_2SO_4 to 100 ml H_2O. When cool, add 900 ml HOAc and mix.

(**c**) *Sodium hydroxide soln.*—Approx. 25%. Dissolve 250 g NaOH in H_2O and dil. to 1 L.

26.066 SEPARATION AND IDENTIFICATION

Place 125 ml oil and 250 ml petr. ether in each of 6 separators. Shake contents of first with 50 ml Soln A and, as soon as layers sep., transfer lower layer to flask contg 250 ml H_2O. Mix, and immediately ext. this dild acid soln by passing successively thru two 500 ml separators, each contg 75 ml petr. ether. Shake vigorously, let layers sep., and discard lower aq. layer. Repeat this procedure

with each of other 5 separators, using same petr. ether to re-ext. colors from the dild acid solns. Combine the 2 petr. ether exts, wash with three 25 ml portions H_2O, and filter.

Ext. combined petr. ether soln with two 25 ml portions Soln *A*. Treat each acid ext. separately by mixing with 150 ml H_2O and re-extg quickly by passing thru two 250 ml separators, each contg 50 ml petr. ether. Combine these petr. ether solns, wash acid-free with 15 ml portions H_2O, and evap. to dryness on steam bath. Do not heat dish after removal of solvent. Residue may contain Ext. D&C Yellow No. 9 or No. 10 (formerly FD&C Yellow No. 3 or No. 4, resp.) and possibly trace of Ext. D&C Orange No. 4 (formerly FD&C Orange No. 2) if latter dye was originally present in large quantities. Identify color spectrophotometrically as in Chap. **35**.

Shake contents of first separator contg the dild oil with 25 ml Soln *B*, let sep. 20 min., and then transfer lower layer to flask contg 200 ml 25% NaOH. Mix, and add 200 ml H_2O. Cool, and remove color from this alk. soln by passing successive 100 ml portions thru two 250 ml separators, each contg 75 ml petr. ether. Discard extd alk. soln. Continue this Soln *B* treatment of oil in other 5 separators, and finally combine the 2 petr. ether exts. Wash with three 50 ml portions H_2O and ext. with two 20 ml portions Soln *B*, letting layers sep. 5 min. Drain lower layers into flask contg 300 ml 25% NaOH, mix, and add 300 ml H_2O. Cool, and remove color from this alk. soln by passing successive 100 ml portions thru 2 separators, each contg 75 ml petr. ether. Combine petr. ether solns, wash free from alkali with H_2O, transfer to evapg dish, and remove solvent on steam bath. Residue may contain Ext. D&C Orange No. 4 and D&C Green No. 6. Remove former by dissolving in 3–5 ml portions 60% alcohol and filtering each portion thru small paper. Identify color spectrophotometrically as in Chap. **35**.

Dissolve residue on paper in 2–5 ml portions petr. ether, collecting filtrate in original evapg dish. Remove solvent on steam bath. Blue residue indicates D&C Green No. 6. Dissolve residue in 15 ml alcohol and 10 ml H_2O, and add 0.5 ml HOAc. Identify color spectrophotometrically as in Chap. **35**.

Pink color in the various acid exts usually indicates coal-tar dye. However, corn oil sometimes produces faint pink color in these exts. This is readily differentiated from coal-tar colors spectrophotometrically. Chlorophyll may appear as green scum at interface in acid exts and as green residue on papers after filtration of petr. ether solns.

ANTIOXIDANTS

Propyl Gallate (*32*)—First Action

26.067 REAGENTS

(**a**) *Petroleum ether reagent.*—Mix 1 vol. 30–60° petr. ether (**13.074** or equiv.) with 3 vols 60–100° petr. ether (Skellysolve B and H have been found satisfactory) and shake mixt. 5 min. with $\frac{1}{10}$ its vol. H_2SO_4. Discard acid layer, wash several times with H_2O, then once with 1% NaOH soln, and then again with H_2O until washings are substantially neutral. Discard all washings and distill petr. ether in all-glass app.

(**b**) *Ammonium acetate solns.*—1.25%, 1.67%, and 10% w/v aq. solns. Soln contg 1.67% NH_4OAc in 5% alcohol may also be required.

(**c**) *Ferrous tartrate reagent.*—Dissolve 0.100 g $FeSO_4.7H_2O$ and 0.500 g Rochelle salt ($NaKC_4H_4O_6.4H_2O$) in H_2O and dil. to 100 ml. Reagent must be used within 3 hr of prepn.

(**d**) *Propyl gallate std soln.*—50 mmg/ml. Dissolve 50 mg propyl gallate in H_2O and dil. to 1 L with H_2O.

26.068 PREPARATION OF STANDARD CURVE

Place at least 7 aliquots of std soln, **26.067**(**d**), covering range from 50 to 1000 mmg, in 50 ml g-s. erlenmeyers. Add exactly 2.5 ml 10% NH_4OAc to each flask, dil. to exactly 24 ml with H_2O, and pipet 1 ml ferrous tartrate reagent into each flask. Let solns stand at least 3 min. Measure absorbances at 540 mμ relative to soln contg 20 ml 1.25% NH_4OAc soln, 4 ml H_2O, and 1 ml ferrous tartrate soln. Plot mmg propyl gallate against absorbance.

26.069 DETERMINATION

Dissolve 40 g of the fat or oil in the petr. ether reagent and dil. to 250 ml with this reagent. (Gentle warming may be necessary to obtain complete soln.) Pipet 100 ml of the fat soln into 250 ml separator. Ext. fat soln with 20 ml of the aq. 1.67% NH_4OAc soln by continuously inverting separator 2.5 min. After phases sep. completely, drain aq. layer into 100 ml vol. flask, being careful not to let any oil droplets fall into flask. (Some shortenings show strong tendency to emulsify during aq. extn. To prevent this emulsification, add 2 ml *n-octanol* to the fat soln aliquot before beginning extn and use the 1.67% NH_4OAc soln in 5% alcohol for extn in place of the aq. soln. This procedure need be used only when usual method fails.)

Repeat extn twice with 20 ml portions 1.67% NH_4OAc soln, combining aq. layers in the vol. flask. Finally, ext. fat soln with 15 ml H_2O for 30 sec. and combine aq. layer with previous wash-

ings. Let layers sep. completely after each washing. Add exactly 2.5 ml 10% NH_4OAc soln to combined exts in vol. flask and dil. to vol. with H_2O. This soln now contains 1.25% NH_4OAc. Filter thru dry rapid paper to remove any turbidity. (Colors must be developed on same day ext. is prepd. If combined exts stand more than several hr, yellow color may develop, and solns must be discarded.)

Pipet aliquot of ext., not >20 ml, into 50 ml g-s. erlenmeyer. Dil. to 20 ml with 1.25% NH_4OAc soln. Add exactly 4 ml H_2O and pipet 1 ml ferrous tartrate reagent into flask. Mix well, and measure absorbance at 540 mμ relative to soln contg 20 ml 1.25% NH_4OAc soln, 4 ml H_2O, and 1 ml ferrous tartrate reagent. Calc. amount of propyl gallate from std curve.

FLAXSEED

Oil by Refraction (*33*)—Official

26.070 PREPARATION OF STANDARD SOLVENT

Prep. mixt. of ca 74% α-chloronaphthalene (Halowax) and 26% α-bromonaphthalene by wt, and carefully adjust composition of mixt. to refractive index of 1.63940 at 25.0°. (If temp. regulating device is available, detn of refractive index is simplified by passing H_2O at exactly 25.0° thru H_2O jacket of refractometer. Equally satisfactory results may be obtained, however, by using H_2O at room temp. and making necessary correction. For above mixt. this correction in refractive index is 0.00045/1°, to be added to reading if temp. is >25.0° and subtracted if temp. is <25°. It is important that all H_2O-jacket temp. readings be made to nearest 0.1°.)

Keep soln in Pb- or g.-s. dark bottle and away from direct sunlight. (Refractive index should remain constant for long period of time, but it is advisable to check it from time to time.)

26.071 PREPARATION OF SAMPLE

Obtain representative sample of ca 25 g clean seed either by hand quartering original sample or by use of mechanical sampling device. Grind material to such fineness that, after extn with ether, 95% of sample passes thru No. 40 sieve. (Motor-driven experimental roller flouring mill with 6×6″ rolls, 40 corrugations to inch, has been found satisfactory. Rolls should have speed differential of 9:7, and faster roll should have speed of ca 900 rpm.) (*See* Fig. 57.)

26.072 DETERMINATION

Weigh accurately 2.5 g finely ground, well-mixed sample and transfer to clean 3″ porcelain mortar preheated to ca 70° in oven or on elec. hot plate at low heat. Add ca 1 g reagent quality sea sand or similar abrasive and exactly 5 ml of the std mixt. of Halowax and α-bromonaphthalene. (Since this mixt. has high sp. gr. it is important to measure its vol. accurately; this is best accomplished with accurately calibrated 5 ml pipet having delivery time not <15 sec.)

Grind mixt. in mortar vigorously 3 min., constantly scraping into bottom particles of meal that are thrown against sides. Filter into test tube thru S&S No. 588 folded paper, or equiv. fat-free paper that yields clear filtrate. When filtrate has cooled to room temp., det. its refractive index at 25.0° to accuracy of ±0.00002. (Dipping type refractometer equipped with interchangeable, H_2O-jacketed, double prism heads, Fig. 58, is recommended.) If reading is made at temp. other than 25.0°, make correction as in **26.070**, using temp. coefficient of 0.00042/1°. Using table, **26.073**, note % oil corresponding to refractive index of filtrate (*uncorrected value* for oil content).

Place ca 2 g ground sample in fine paper filter in glass funnel and pour over it ca 15 ml petr. ether,

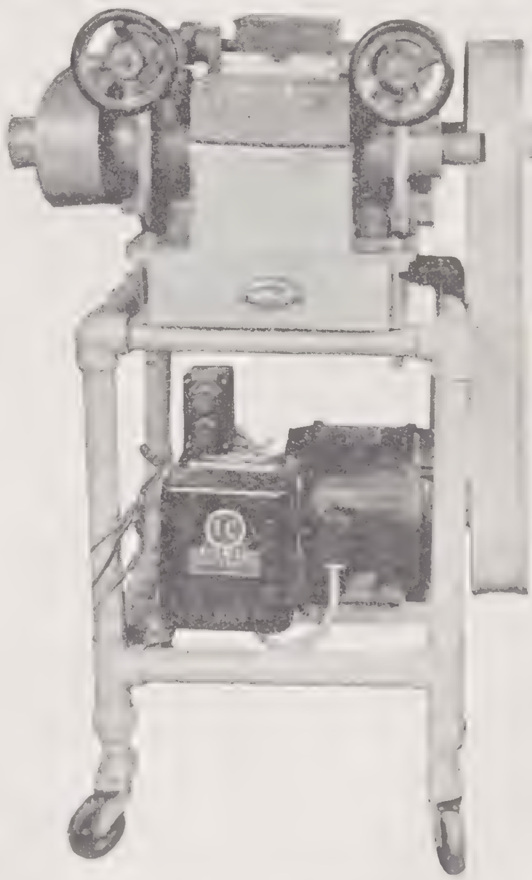


FIG. 57.—ROLLER-TYPE EXPERIMENTAL FLOURING MILL SUITABLE FOR GRINDING FLAXSEED SAMPLES

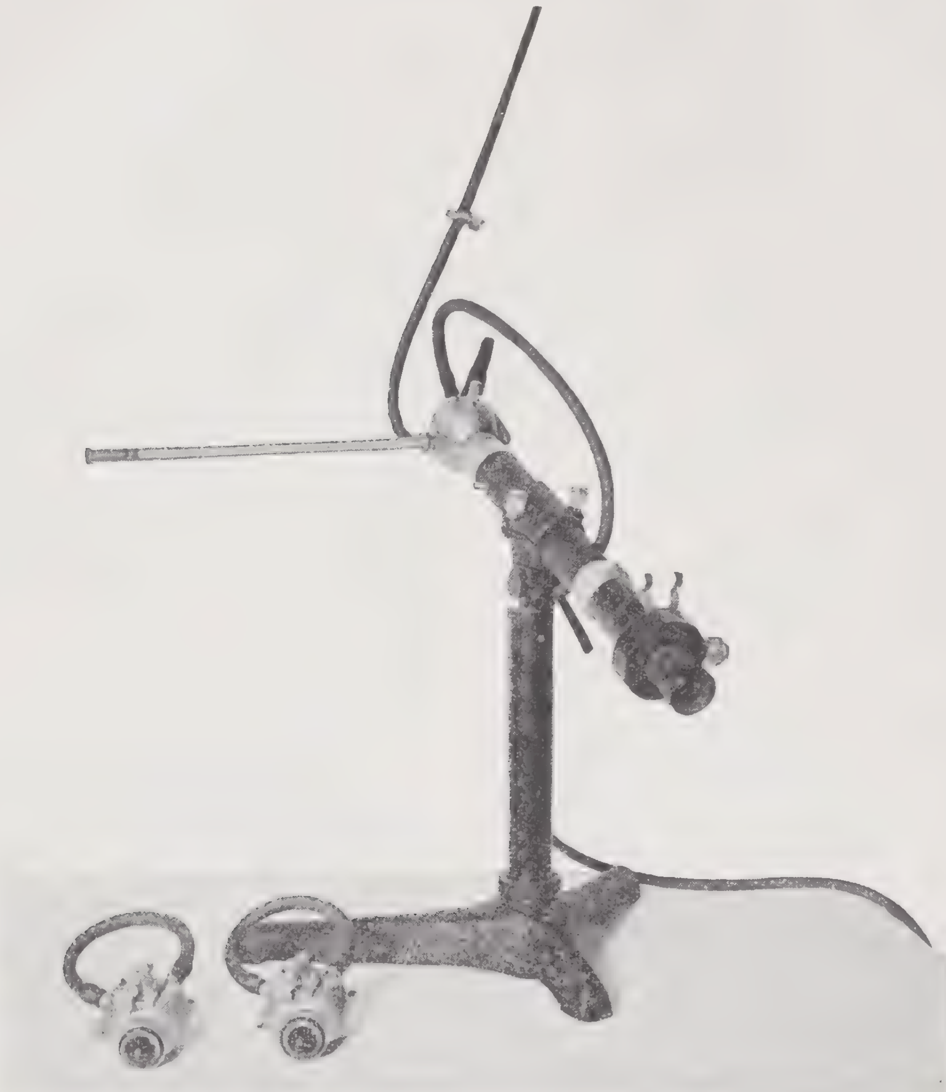


FIG. 58.—DIPPING-TYPE REFRACTOMETER WITH INTERCHANGEABLE DOUBLE PRISM HEADS SUITABLE FOR REFRACTOMETRIC DETERMINATION OF OIL CONTENT OF FLAXSEED

collecting clear filtrate in small, shallow evapg dish. Carefully evap. ether on steam bath or hot plate at low heat, keep dish in oven 20 min. at 105°, and cool to room temp. (If preferred, prep. oil sample by pressing small sample of the ground seed in laboratory hydraulic press and filtering oil obtained if it is not entirely clear.) Det. refractive index of oil at 25.0°. Temp. coefficient for pure oil is 0.000357/1.0°, to be added if temp. at which reading is taken is >25.0° and subtracted if <25°.

From refractive index value of oil subtract value 1.47780 (refractive index at 25.0° of composite sample of oil used in obtaining data, **26.073**). Using this difference, det. from table, **26.074**, correction to be applied to uncorrected value for oil content as detd above. If difference is positive, add correction; if negative, subtract.

26.073 *Conversion table for determining percentage of oil in flaxseed from refractive index of Halowax, α-bromonaphthalene extract at 25.0°*

$n^{25}D$	OIL	$n^{25}D$	OIL	$n^{25}D$	OIL	$n^{25}D$	OIL
	%		%		%		%
1.61837	28.0	1.61554	32.5	1.61279	37.0	1.61012	41.5
1.61831	28.1	1.61548	32.6	1.61273	37.1	1.61006	41.6
1.61824	28.2	1.61542	32.7	1.61267	37.2	1.61000	41.7
1.61818	28.3	1.61535	32.8	1.61261	37.3	1.60995	41.8
1.61811	28.4	1.61529	32.9	1.61255	37.4	1.60989	41.9
1.61805	28.5	1.61523	33.0	1.61249	37.5	1.60983	42.0
1.61799	28.6	1.61517	33.1	1.61243	37.6	1.60977	42.1
1.61792	28.7	1.61511	33.2	1.61237	37.7	1.60971	42.2
1.61786	28.8	1.61504	33.3	1.61231	37.8	1.60966	42.3
1.61779	28.9	1.61498	33.4	1.61225	37.9	1.60960	42.4
1.61773	29.0	1.61492	33.5	1.61219	38.0	1.60954	42.5
1.61767	29.1	1.61486	33.6	1.61213	38.1	1.60948	42.6
1.61760	29.2	1.61480	33.7	1.61207	38.2	1.60942	42.7
1.61754	29.3	1.61473	33.8	1.61201	38.3	1.60937	42.8
1.61748	29.4	1.61467	33.9	1.61195	38.4	1.60931	42.9
1.61742	29.5	1.61461	34.0	1.61189	38.5	1.60925	43.0
1.61735	29.6	1.61455	34.1	1.61183	38.6	1.60919	43.1
1.61729	29.7	1.61449	34.2	1.61177	38.7	1.60913	43.2
1.61723	29.8	1.61443	34.3	1.61171	38.8	1.60908	43.3
1.61716	29.9	1.61437	34.4	1.61165	38.9	1.60902	43.4
1.61710	30.0	1.61431	34.5	1.61159	39.0	1.60896	43.5
1.61704	30.1	1.61424	34.6	1.61153	39.1	1.60890	43.6
1.61697	30.2	1.61418	34.7	1.61147	39.2	1.60884	43.7
1.61691	30.3	1.61412	34.8	1.61141	39.3	1.60879	43.8
1.61685	30.4	1.61406	34.9	1.61135	39.4	1.60873	43.9
1.61679	30.5	1.61400	35.0	1.61130	39.5	1.60867	44.0
1.61672	30.6	1.61394	35.1	1.61124	39.6	1.60861	44.1
1.61666	30.7	1.61388	35.2	1.61118	39.7	1.60856	44.2
1.61660	30.8	1.61382	35.3	1.61112	39.8	1.60850	44.3
1.61653	30.9	1.61376	35.4	1.61106	39.9	1.60844	44.4
1.61647	31.0	1.61370	35.5	1.61100	40.0	1.60839	44.5
1.61641	31.1	1.61363	35.6	1.61094	40.1	1.60833	44.6
1.61635	31.2	1.61357	35.7	1.61088	40.2	1.60827	44.7
1.61628	31.3	1.61351	35.8	1.61082	40.3	1.60821	44.8
1.61622	31.4	1.61345	35.9	1.61076	40.4	1.60816	44.9
1.61616	31.5	1.61339	36.0	1.61071	40.5	1.60810	45.0
1.61610	31.6	1.61333	36.1	1.61065	40.6	1.60804	45.1
1.61604	31.7	1.61327	36.2	1.61059	40.7	1.60799	45.2
1.61597	31.8	1.61321	36.3	1.61053	40.8	1.60793	45.3
1.61591	31.9	1.61315	36.4	1.61047	40.9	1.60787	45.4
1.61585	32.0	1.61309	36.5	1.61041	41.0	1.60782	45.5
1.61579	32.1	1.61303	36.6	1.61035	41.1	1.60776	45.6
1.61573	32.2	1.61297	36.7	1.61029	41.2	1.60770	45.7
1.61566	32.3	1.61291	36.8	1.61024	41.3	1.60764	45.8
1.61560	32.4	1.61285	36.9	1.61018	41.4		

26.074 *Corrections* to be applied to results obtained in analysis of flaxseed for oil content by refractometric method*

[Values to be added when ($n_D^{25} - 1.4778$) is positive, subtracted when ($n_D^{25} - 1.4778$) is negative]
(Corrections in terms of % oil indicated)

$n_D^{25} - 1.4778$	28*	29*	30*	31*	32*	33*	34*	35*	36*	37*	38*	39*	40*	41*	42*	43*	44*	45*	46*	47*	48*
0.0001...	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.03	0.03	0.03	0.03	0.03
.0002...	.03	.03	.03	.03	.04	.04	.04	.04	.04	.04	.04	.04	.05	.05	.05	.05	.05	.05	.05	.05	.06
.0003...	.05	.05	.05	.05	.05	.06	.06	.06	.06	.06	.06	.07	.07	.07	.07	.07	.08	.08	.08	.08	.08
.0004...	.06	.06	.07	.07	.07	.07	.08	.08	.08	.08	.09	.09	.09	.09	.10	.10	.10	.10	.11	.11	.11
.0005...	.08	.08	.08	.09	.09	.09	.10	.10	.10	.11	.11	.11	.11	.12	.12	.12	.13	.13	.13	.14	.14
.0006...	.09	.10	.10	.10	.11	.11	.11	.12	.12	.13	.13	.13	.14	.14	.15	.15	.15	.16	.16	.16	.17
.0007...	.11	.11	.12	.12	.12	.13	.13	.14	.14	.15	.15	.16	.16	.17	.17	.17	.18	.18	.19	.19	.20
.0008...	.12	.13	.13	.14	.14	.15	.15	.16	.16	.17	.17	.18	.18	.19	.19	.20	.20	.21	.21	.22	.22
.0009...	.14	.14	.15	.15	.16	.17	.17	.18	.18	.19	.19	.20	.21	.21	.22	.22	.23	.23	.24	.25	.25
.0010...	.15	.16	.17	.17	.18	.19	.19	.20	.20	.21	.22	.22	.23	.24	.24	.25	.26	.26	.27	.27	.28
.0011...	.17	.17	.18	.19	.20	.20	.21	.22	.22	.23	.24	.25	.25	.26	.27	.27	.28	.29	.29	.30	.31
.0012...	.18	.19	.20	.21	.21	.22	.23	.24	.24	.25	.26	.27	.27	.28	.29	.30	.31	.31	.32	.33	.34
.0013...	.20	.21	.22	.22	.23	.24	.25	.26	.27	.27	.28	.29	.30	.31	.31	.32	.33	.34	.35	.36	.36
.0014...	.21	.22	.23	.24	.25	.26	.27	.28	.29	.29	.30	.31	.32	.33	.34	.35	.36	.37	.37	.38	.39
.0015...	.23	.24	.25	.26	.27	.28	.29	.30	.31	.32	.32	.33	.34	.35	.36	.37	.38	.39	.40	.41	.42
.0016...	.24	.25	.27	.28	.28	.30	.31	.32	.33	.34	.35	.36	.37	.38	.39	.40	.41	.42	.43	.44	.45
.0017...	.26	.27	.28	.29	.30	.31	.32	.33	.35	.36	.37	.38	.39	.40	.41	.42	.43	.44	.45	.47	.48
.0018...	.28	.29	.30	.31	.32	.33	.34	.35	.37	.38	.39	.40	.41	.42	.44	.45	.46	.47	.48	.49	.50
.0019...	.29	.30	.32	.33	.34	.35	.36	.37	.39	.40	.41	.42	.44	.45	.46	.47	.48	.50	.51	.52	.53
.0020...	.31	.32	.33	.34	.36	.37	.38	.39	.41	.42	.43	.45	.46	.47	.48	.50	.51	.52	.53	.55	.56
.0021...	.32	.33	.35	.36	.37	.39	.40	.41	.43	.44	.45	.47	.48	.50	.51	.52	.54	.55	.56	.58	.59
.0022...	.34	.35	.37	.38	.39	.41	.42	.43	.45	.46	.48	.49	.50	.52	.53	.55	.56	.57	.59	.60	.62
.0023...	.35	.37	.38	.40	.41	.43	.44	.45	.47	.48	.50	.51	.53	.54	.56	.57	.59	.60	.61	.63	.64
.0024...	.37	.38	.40	.41	.43	.44	.46	.47	.49	.50	.52	.54	.55	.57	.58	.60	.61	.63	.64	.66	.67
.0025...	.38	.40	.42	.43	.45	.46	.48	.49	.51	.53	.54	.56	.57	.59	.61	.62	.64	.65	.67	.69	.70
.0026...	.40	.41	.43	.45	.46	.48	.50	.51	.53	.55	.56	.58	.60	.61	.63	.64	.66	.68	.69	.71	.73
.0027...	.41	.43	.45	.46	.48	.50	.52	.53	.55	.57	.58	.60	.62	.64	.65	.67	.69	.70	.72	.74	.76
.0028...	.43	.45	.46	.48	.50	.52	.53	.55	.57	.59	.60	.62	.64	.66	.68	.69	.71	.73	.75	.77	.78
.0029...	.44	.46	.48	.50	.52	.54	.55	.57	.59	.61	.63	.65	.66	.68	.70	.72	.74	.76	.77	.79	.81
.0030...	.46	.48	.50	.52	.53	.55	.57	.59	.61	.63	.65	.67	.69	.71	.73	.74	.76	.78	.80	.82	.84

* Per cent oil as determined from 26.073.

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27. Preservatives and Artificial Sweeteners

27.001 **ANTIOXIDANTS—**

See Chapter **26**

BENZOIC ACID

Qualitative Test

27.002 *Preliminary Test—Official*

Ext. benzoic acid as in **27.073** or **27.075.** If appreciable benzoic acid is present, it will crystallize from ether in shining leaflets having characteristic odor on warming. Dissolve cryst. deposit in hot H_2O, divide into 2 portions, and test as in **27.003** or **27.004.** Deposit may also be purified as in **27.073**(**c**) and m.p. detd.

27.003 *Ferric Chloride Test—Official*

Make soln, **27.002,** alk. with few drops of NH_4OH, expel excess NH_3 by evapn, dissolve residue in few ml hot H_2O, filter if necessary, and add few drops *aq. 0.5% $FeCl_3$ soln.* Salmon-color ppt of ferric benzoate indicates presence of benzoic acid.

27.004 *Modified Mohler Test (1)—Official*

(Presence of phthln interferes)

Add to aq. soln, **27.002,** 1 or 2 drops ca 10% NaOH soln and evap. to dryness. To residue add 5–10 drops H_2SO_4 and small crystal KNO_3. Heat 10 min. in glycerol bath at 120–130° (must not be >130°). Cool, add 1 ml H_2O, and make distinctly ammoniacal. Boil soln to decompose any NH_4NO_2 that may form. Cool, and add drop of fresh, *colorless $(NH_4)_2S$ soln,* but do not let layers mix. Red-brown ring indicates benzoic acid. On mixing, color diffuses thruout liquid, and on heating finally changes to greenish-yellow. This change differentiates benzoic acid from salicylic acid or cinnamic acid. Salicylic and cinnamic acids form colored compounds that are not destroyed by heating.

Quantitative Methods

Titrimetric Method—Official

27.005 PREPARATION OF SAMPLE

(**a**) *General method.*—Mix sample thoroly, grinding if solid or semi-solid. Transfer 150 ml or 150 g to 500 ml vol. flask, add enough pulverized NaCl to sat. H_2O in sample, make alk. to litmus paper with 10% NaOH soln or with milk of lime (1 part powd. recently slaked $Ca(OH)_2$ suspended in 3 parts H_2O), and dil. to mark with satd NaCl soln. Shake thoroly, let stand at least 2 hr, shaking frequently, and filter. If sample contains large quantities of fat, portions of which may contaminate filtrate, add few ml of the NaOH soln to filtrate and ext. with ether before proceeding as in **27.006.** If alcohol is present, proceed as in (**d**). If sample contains large quantities of matter precipitable by NaCl soln, proceed as in (**e**).

(**b**) *Catsup.*—Add 15 g pulverized NaCl to 150 g sample, and transfer mixt. to 500 ml vol. flask, rinsing with ca 150 ml satd NaCl soln. Make slightly alk. to litmus paper with 10% NaOH soln and fill to mark with satd NaCl soln. Let stand at least 2 hr, shaking frequently. Squeeze thru heavy muslin bag and filter.

(**c**) *Jellies, jams, preserves, and marmalades.*—Digest 150 g sample in ca 300 ml satd NaCl soln. Add 15 g pulverized NaCl. Make alk. to litmus paper with milk of lime. Transfer to 500 ml vol. flask and dil. to mark with satd NaCl soln. Let stand at least 2 hr, shaking frequently; centrifuge if necessary, and filter.

(**d**) *Cider containing alcohol, and similar products.*—Make 250 ml sample alk. to litmus paper with 10% NaOH soln and evap. on steam bath to ca 100 ml. Transfer to 250 ml vol. flask, add 30 g pulverized NaCl, and shake until dissolved. Dil. to original vol. of 250 ml with satd NaCl soln; let stand at least 2 hr, shaking frequently, and filter.

(**e**) *Salted or dried fish.*—Wash 50 g ground sample into 500 ml vol. flask with H_2O. Make slightly alk. to litmus paper with 10% NaOH soln and dil. to mark with H_2O. Let stand at least 2 hr, shaking frequently, and filter. Pipet as large a measured portion of filtrate as possible (at least 300 ml) into second 500 ml vol. flask, and add 30 g pulverized NaCl for each 100 ml soln. Shake until NaCl dissolves and dil. to mark with satd NaCl soln. Mix thoroly, and filter off pptd protein and other extraneous matter.

27.006 DETERMINATION

Pipet 100–200 ml filtrate, **27.005,** into separator. Neutralize to litmus paper with HCl (1+3) and add 5 ml excess. With salted fish, protein usually ppts on acidifying, but ppt does not interfere with extn. Ext. carefully with $CHCl_3$, using

successive portions of 70, 50, 40, and 30 ml. To avoid formation of emulsion, shake cautiously each time, using rotary motion. $CHCl_3$ layer usually seps readily after standing few min. If emulsion forms, break it by stirring $CHCl_3$ layer with glass rod, by drawing off into second separator and giving 1 or 2 sharp shakes from one end of separator to other, or by centrifuging few min. As this is progressive extn, draw off carefully as much clear $CHCl_3$ soln as possible after each extn, but do not draw off any of emulsion with $CHCl_3$ layer. If this precaution is taken $CHCl_3$ ext. need not be washed.

Transfer combined $CHCl_3$ exts to porcelain evapg dish, rinse container several times with few ml $CHCl_3$, and evap. to dryness at room temp. in current of dry air.

Ext. may also be transferred from separator to 300 ml erlenmeyer and separator rinsed with three 5–10 ml portions $CHCl_3$. Distill very slowly at low temp. to ca $\frac{1}{4}$ original vol. Transfer residue to porcelain evapg dish, rinsing flask with three 5–10 ml portions $CHCl_3$, and evap. to dryness at room temp. in current of dry air.

Dry residue overnight (or until no odor of HOAc can be detected if product is catsup) in desiccator contg H_2SO_4. Dissolve residue of benzoic acid in 30–50 ml alcohol neutral to phthln; add ca $\frac{1}{4}$ this vol. of H_2O and 1 or 2 drops phthln; and titr. with 0.05*N* NaOH. 1 ml 0.05*N* NaOH = 0.0072 g anhyd. Na benzoate.

Spectrophotometric Method (2)—First Action

(Applicable to catsup, other tomato products, jams, jellies, beverages contg small amounts of alcohol, soft drinks, and fruit juices. Not applicable to solids.)

27.007 PREPARATION OF STANDARD CURVE

Prep. soln of benzoic acid in ether contg 50 mg/L. Det. absorbance of this soln in well stoppered cuvette in Beckman DU or recording spectrophotometer between 265 and 280 mμ in 1 mμ intervals. Plot absorbance against wavelength and record wavelength of min. at ca 267.5 mμ as point *A*, other min. at ca 276.5 mμ as point *C*, and highest max. at ca 272 mμ as point *B*.

Prep. solns of benzoic acid in ether contg 20, 40, 60, 80, 100, and 120 mg/L. Det. absorbances of these solns in well stoppered cuvette in spectrophotometer at points *A*, *B*, and *C*. For each concn, average absorbance at *A* and *C* and subtract this value from absorbance at *B*. Plot difference against concn.

27.008 PREPARATION OF SAMPLE

Mix sample thoroly. Transfer 10 g or 10 ml to separator and dil. to 200 ml with satd NaCl soln. Make soln definitely acid to litmus with HCl and mix well.

27.009 DETERMINATION

Ext. prepd soln with 70, 50, 40, and 30 ml portions of ether, shaking well to insure complete extn. (Break emulsions by standing, stirring, or centrifuging.) Drain and discard aq. phase. Wash combined ether exts with 50, 40, and 30 ml portions HCl (1+1000) and discard HCl washings. (If ext. requires no purification, proceed to next par.) Ext. ether soln with 50, 40, 30, and 20 ml portions 0.1% NH_4OH and discard ether. Neutralize combined NH_4OH exts with HCl and add 1 ml excess. Ext. acidified soln with 70, 50, 40, and 30 ml ether.

Dil. combined ether exts to 200 ml with ether and det. absorbance in well stoppered cuvette in spectrophotometer at wavelengths *A*, *B*, and *C*, dilg with ether if necessary to obtain optimum concn of 20–120 mg/L. Average absorbances at *A* and *C* and subtract this value from absorbance at *B*. Det. concn benzoic acid from std curve, correcting for dilns. Benzoic acid × 1.18 = Na benzoate.

Conduct detn similarly on benzoate-free sample of product and det. absorbances in region 265 to 280 mμ at 1 mμ intervals. If curve is straight line in this region, method is applicable to this product.

BORIC ACID AND BORATES

27.010 Qualitative Test *(3)*—**Official**

(a) *Preliminary test.*—Acidify sample with HCl (7 ml acid to each 100 ml sample). Heat solid or pasty samples with enough H_2O to make sufficiently fluid before acidifying. Immerse strip of turmeric paper in acidified liquid, and let paper dry spontaneously. If $Na_2B_4O_7$ or H_3BO_3 is present, paper acquires characteristic red color, changed by NH_4OH to dark blue-green, but restored by acid.

(b) *Confirmatory test.*—Make ca 25 g sample decidedly alk. with lime-H_2O or milk of lime and evap. to dryness on steam bath. Ignite dry residue at low red heat until org. matter is thoroly charred. Cool, digest with ca 15 ml H_2O, and add HCl dropwise until soln is distinctly acid. Immerse piece of turmeric paper in soln and dry without heat. In presence of $Na_2B_4O_7$ or H_3BO_3 color change will be same as in (a).

Semiquantitative Method *(3)*—Official

(Applicable to meat)

27.011 REAGENTS

(a) *Turmeric paper.*—Add 100 ml 80% alcohol to 1.5–2.0 g turmeric powder in 250 ml g-s. erlenmeyer. Shake 5 min. and filter. Dip sheets of Whatman No. 2 filter paper into the clear filtrate in flat bottom dish (petri dish). Hang paper to

dry. After 1 hr cut into strips $2\frac{1}{4} \times \frac{3}{8}''$ and store in tightly stoppered container protected from light.

(**b**) *Boric acid std soln.*—Dissolve 1.000 g H_3BO_3 in H_2O and dil. to 100 ml. (1 ml = 10 mg H_3BO_3.)

27.012 PREPARATION OF REFERENCE STANDARDS

Transfer 0.00, 0.10, 0.20, 0.50, 0.75, 1.00, 2.50, and 5.00 ml std H_3BO_3 soln to 15 ml test tubes. Dil. to 10 ml with H_2O and add 0.7 ml HCl. Keep tubes tightly stoppered to prevent evapn. These stds represent 0.00, 0.02, 0.04, 0.10, 0.15, 0.20, 0.50, and 1.00% H_3BO_3 in meat (based on 25 g sample extd with 50 ml H_2O and 10 ml aliquot used for test). Std solns may be stored in Pyrex glass test tubes for >6 months. On long storage, borate is leached from the glass.

27.013 DETERMINATION

Disperse 25 g ground meat in 50 ml H_2O in 125 ml erlenmeyer, using flat-end stirring rod. Cover with watch glass or small funnel. Bring to boil on hot plate (or over medium flame) with agitation. Do not overheat. Cool in ice bath or in beaker of H_2O in refrigerator until fat solidifies (ca 0.5 hr). Filter thru pledget of glass wool. Transfer 10 ml filtrate to 15 ml test tube, add 0.7 ml HCl, stopper, and mix.

Mark identification on end of piece of turmeric paper and dip unmarked end into unknown soln to ½ the length of paper. Quickly remove moistened paper and place on sheet of white filter paper. Flat-tipped forceps are useful in handling paper.

Place freshly prepd std strips of test paper (made by dipping turmeric papers in similar manner into series of std solns) alongside sample turmeric strips.

After at least 1 hr (but <2 hr) at room temp., strips are dry enough for comparison. Good natural light is preferred. Place std strips ca 0.5″ apart on white filter paper background and bring "unknown" sample strips between adjacent stds for close color matching. If color falls between 2 stds, estimate value. Disregard streaks of color that may develop at edge of test strip.

If color intensity is beyond range of stds, repeat test with appropriate diln of meat filtrate; i.e., 5 ml filtrate, 5 ml H_2O, 0.7 ml HCl; and multiply final reading by 2. Use freshly prepd set of std papers with each series of samples tested.

27.014 Quantitative Method (*5*)—**Official**

Make 10–100 g sample (depending upon material and quantity of H_3BO_3 present) distinctly alk. with 10% NaOH soln and evap. to dryness in Pt dish. Ignite residue until org. matter is thoroly charred, avoiding intense red heat; cool. digest with ca 20 ml hot H_2O, and add HCl dropwise until reaction is distinctly acid. Filter into 100 ml vol. flask and wash with little hot H_2O. (Vol. filtrate should be <50–60 ml.) Return filter contg any unoxidized C to Pt dish, make alk. by wetting thoroly with lime-H_2O, dry on steam bath, and ignite to white ash.

Dissolve ash in few ml HCl (1+3) and add to liquid in 100 ml flask, rinsing dish with few ml H_2O. To combined solns add 0.5 g $CaCl_2$ and few drops of phthln, then 10% NaOH soln until permanent light pink is produced. Finally dil. to mark with lime-H_2O, mix, and filter thru dry filter. To 50 ml filtrate add $1N$ H_2SO_4, **2.034(j)**, until pink disappears; then add Me orange, **4.004(g)**, and continue addn of acid until yellow changes to pink. Boil ca 1 min. to expel CO_2. Cool, and carefully add $0.2N$ NaOH until liquid assumes yellow tinge, avoiding excess alkali. (All H_3BO_3 is now in free state with no uncombined H_2SO_4 present.) Add 1–2 g neutral mannitol and few drops phthln, read buret, and again titr. soln with the std NaOH until pink. Add little more mannitol, and if pink disappears, continue addn of the std alkali until pink reappears. Repeat alternate addn of mannitol and std alkali until permanent end point is reached. Vol. of glycerol (neutral to phthln) equal to vol. of soln to be titrd may be substituted for mannitol. 1 ml $0.2N$ NaOH = 0.0124 g H_3BO_3.

CYCLOHEXYLSULFAMATE SALTS

Qualitative Test—First Action

27.015 *Sodium Nitrite Test*

Add 2 g $BaCl_2$ to 100 ml sample or aq. ext., prepd as in **27.072(c)**. Let stand 5 min. and filter. Acidify with 10 ml HCl and add 0.2 g $NaNO_2$. White ppt of $BaSO_4$ indicates presence of cyclohexylsulfamate.

27.016 Quantitative Method (*7*)—**Official**

(Applicable to aq. solns and clear carbonated beverages)

To 100 ml soln contg 10–300 mg Na or Ca cyclohexylsulfamate add 10 ml HCl and 10 ml 10% $BaCl_2$ soln. Stir and let stand 30 min. If ppt forms, filter and wash with H_2O. To filtrate or clear soln add 10 ml 10% $NaNO_2$ soln, stir, cover with watch glass, and heat on steam bath at least 2 hr. Stir up ppt 3 times at 0.5 hr intervals. Remove from steam bath and leave in warm place overnight. Collect ppt on tared gooch, wash, and dry on asbestos mat over flame at least 10 min. Ignite, cool in desiccator, and weigh. Wt $BaSO_4$ $\times 0.8621$ = Na cyclohexylsulfamate; $\times 0.9266$ = Ca cyclohexylsulfamate.

DEHYDROACETIC ACID

(Applicable to cheese)

27.017 Qualitative Test (*6*)—First Action

(**a**) *Salicylaldehyde reagent.*—Dissolve 10 ml salicylaldehyde in alcohol and dil. to 50 ml.

(**b**) *Test.*—Transfer dehydroacetic acid soln remaining in 500 ml vol. flask after quant. detn, **27.018,** to 1 L separator. Add 100–125 ml ether and shake vigorously. Let sep., drain aq. layer, and discard. Drain ether into 125 ml erlenmeyer, taking care not to include any emulsion or H_2O. Evap. ether ext. to dryness on steam bath and dissolve residue in 1 ml ca 0.5*N* NaOH. Pour alk. soln into test tube (do not rinse flask); add 0.5 ml of the alc. salicylaldehyde soln and 1 ml NaOH (1+1). Mix, and place in boiling H_2O bath 5 min. Remove tube from bath, add 2 ml H_2O, and observe color. Include reagent blank and control contg 0.2 or 0.3 mg dehydroacetic acid for comparison. With as little as 10 ppm or less of dehydroacetic acid in cheese, red or orange color is obtained. Intensity of the color is approx. proportional to quantity of dehydroacetic acid present.

Quantitative Method (*6*)—First Action

27.018 DETERMINATION

Weigh 50–60 g cheese to nearest 0.1 g, place in high speed blender, and comminute (covered) with 80 ml $CHCl_3$ 3 min., scraping down walls and cover once during operation. Place filter paper on 2–3″ diam. fritted glass büchner (if fritted glass funnel is not available, use ordinary büchner), transfer mixt. to funnel with spatula, and filter with suction. Return cake and paper to blender, add 80 ml $CHCl_3$, blend 1 min., and refilter into same flask. Use fresh paper for each filtration. Repeat extn and filtration for third time with 80 ml portion $CHCl_3$. Wash sides of filter and cake once with 25 ml $CHCl_3$. Greater portion of the $CHCl_3$ may be removed if cheese cake is compressed.

Transfer combined $CHCl_3$ filtrates to 500 ml separator. Rinse filter flask with 2 small portions $CHCl_3$ and add to separator. Ext. $CHCl_3$ soln with ca 33 ml ca 0.5*N* NaOH. Transfer $CHCl_3$ layer to 600 ml beaker and aq. layer to 300 ml erlenmeyer. Return $CHCl_3$ to separator and repeat above alk. extn twice. Emulsion may be formed during extn, but most of it will break on standing. Transfer emulsified layer to alk. soln only in final extn. Acidify alk. ext. with 70 ml ca 1*N* HCl, and rapidly aerate for such time as required to remove dissolved $CHCl_3$ (5–10 min.). To check complete removal of $CHCl_3$, smell top of flask while aerating. Be sure to remove all $CHCl_3$ by aeration, or low values will be obtained. Filter soln thru medium or fine porosity fritted glass funnel fitted with filter paper and dil. to vol. with H_2O in 500 ml vol. flask. If soln is turbid, clarify by refiltering thru fine filter or asbestos pad.

Prep. reagent blank by extg 250 ml $CHCl_3$ with the alkali, adding acid to ext., aerating, and dilg to vol. with H_2O. Place portion of reagent blank in one cell and portion of sample soln in another. Det. absorbance of the soln at 307 mμ with Beckman DU spectrophotometer or equiv. Dil. sample soln if necessary to obtain readings in range covered by std curve. (Ordinary range of diln for absorbance readings is from no diln to diln of 1+5).

27.019 PREPARATION OF STANDARD CURVE

To prep. std curve use fresh dehydroacetic acid soln, as low readings are obtained from older solns. Weigh exactly 100 mg *dehydroacetic acid* (Eastman or equiv.) and transfer to 100 ml vol. flask. Dissolve in ca 50 ml H_2O+4 ml ca 0.5*N* NaOH. Dil. to vol. with H_2O and mix. Pipet 1.0, 3.0, and 5.0 ml (1.0, 3.0, and 5.0 mg dehydroacetic acid) aliquots of this stock soln into sep. 500 ml vol. flasks. To each add equiv. of ca 100 ml ca 0.5*N* NaOH and 70 ml ca 1*N* HCl, dil. to vol., and mix. Det. absorbance of solns at 307 mμ, using reagent blank prepd as above. Plot absorbance against mg dehydroacetic acid/500 ml prepd soln. Calc. dehydroacetic acid to ppm:

$$\text{ppm} = (\text{mg}/500\ \text{ml}) \times 1000/\text{wt sample}.$$

DULCIN

27.020 Preparation of Sample—First Action

Ext. 100 ml sample (made alk. with 10% NaOH soln, if necessary), or of alk. aq. ext., prepd as in **27.072(c)**, with two or three 50 ml portions ether. Divide ether ext. equally between 2 porcelain dishes, let ether evap. at room temp., and dry residues in oven at 110°.

Qualitative Tests

27.021 *Denigès-Tourrou Test (8)—First Action*

Moisten dry residue, **27.020,** with HNO_3 and add 1 drop H_2O. Presence of dulcin is indicated by formation of orange or brick-red ppt.

27.022 *Modified LaParola-Mariani Test (9)—Official*

Expose dry residue, **27.020,** to HCl gas for 5 min. and add 1 drop *anisaldehyde*. Presence of dulcin is indicated by development of orange-red to blood-red color. Presence of 25 mg/L or kg original sample can usually be detected by this test.

27.023 Quantitative Method (*10*)—**First Action**

(Applicable to non-alcoholic beverages)

Pipet 50 ml sample into separator. If 5-nitro-2-propoxyaniline (P-4000) is present, ext. with four 50 ml portions petr. ether, shaking 2 min. each time, and discard petr. ether. Make aq. phase alk. to litmus with 10% NaOH soln and ext. with four 100 ml portions ether, shaking 2 min. each time. Combine exts, wash with 10 ml H_2O, and discard H_2O. Evap. ether in 400 ml beaker and dry residue 30 min. at 110°.

Dissolve residue in ca 50 ml redistd EtOAc, transfer to 100 ml vol. flask, dil. to vol. with 4 or 5 washings of the EtOAc, and mix. Read absorbance of soln in spectrophotometer at 294 mμ against redistd EtOAc. Det. quantity of dulcin in final soln from previously prepd std curve and calc. to mg/L.

SOLUBLE FLUORIDES

Qualitative Tests

27.024 *Hydrofluoric Acid Test* (*11*)—*Official*

(**a**) *Not applicable in presence of silicates.*—After thoroly mixing sample transfer to beaker 150 ml, or equiv. quantity of aq. ext. in case of solid foods, and boil, adding 5 ml *10% K_2SO_4 soln* and 10 ml *10% $Ba(OAc)_2$ soln*. Collect ppt in compact mass (centrifuge may be used advantageously) and wash upon small filter. Transfer to Pt crucible and ignite.

Dip carefully cleaned glass plate, while hot, in mixt. of equal parts of *carnaüba wax* and *paraffin*, and let cool. Make distinctive mark thru the wax with sharp instrument, taking care not to scratch surface of glass.

Add few drops H_2SO_4 to residue in crucible and cover crucible with the waxed plate, having mark over center of crucible and making sure that edge of crucible is in close contact with plate. Keep top surface of plate cool, and heat crucible 1 hr at as high temp. as practicable without melting wax (elec. stove gives most satisfactory form of heat). If fluorides are present, distinct etching is apparent on exposed glass.

(**b**) *Applicable in presence of silicates.*—Mix small quantity of pptd SiO_2 with pptd BaF_2, (**a**), and proceed as in **27.028** or **27.029**. (This method is valuable for foods which contain considerable quantity of SiO_2 in the ash. Under these circumstances H_2SO_4 liberates SiF_4, which would escape detection in (**a**).)

Quenching of Aluminum 8-Hydroxyquinolate Fluorescence—First Action

27.025 REAGENTS

(**a**) *Aluminum soln.*—Dissolve 2.22 g $AlNH_4(SO_4)_2.12H_2O$ in H_2O, add 3 drops HCl, and dil. to 250 ml with H_2O.

(**b**) *Oxine reagent.*—Dissolve enough 8-hydroxyquinoline in 2N HOAc to make 5% soln. 1 ml of this soln is equiv. to ca 5 ml of the Al soln.

(**c**) *Ammonium acetate soln.*—Dissolve 77 g NH_4OAc in H_2O and dil. to 500 ml with H_2O.

(**d**) *Aluminum 8-hydroxyquinolate.*—Warm 250 ml of the Al soln to 50–60° and add excess of oxine reagent. Add NH_4OAc soln slowly until permanent ppt forms. Then add 20–25 ml more to insure complete pptn. Let ppt settle and filter thru fritted glass crucible. Wash ppt well with at least seven or eight 30 ml portions cold H_2O and dry at 120–140°. Store in desiccator.

(**e**) *Chloroform soln of aluminum 8-hydroxyquinolate.*—Dissolve Al oxine in $CHCl_3$ to prep. 0.5 mg/ml soln. Prep. daily.

(**f**) *Sulfuric acid.*—Concd. If blank detn reveals presence of F, purify as in **24.028**(**c**), dilg and boiling 3 times.

27.026 TEST

Proceed as in **27.024**(**a**), adding 3 ml HOAc to soln in addn to K_2SO_4 and $Ba(OAc)_2$ solns. Transfer ignited residue to small porcelain crucible (5 ml or smaller).

Wet piece of filter paper with the $CHCl_3$ soln of Al oxine in spot larger in diam. than top of crucible and let air-dry. Add H_2SO_4 to cover ash, crimp paper over crucible edge, and put wt (e.g., beaker) on paper. Heat crucible covered with paper 5 min. at 50–60°. Observe paper under ultraviolet light. In presence of F, fluorescence of the Al oxine is quenched in area of spot over crucible. Limit of identification is ca 0.05 mg F. Conduct blank detn on H_2SO_4.

INSOLUBLE FLUORIDES

(Fluoborates, fluosilicates, etc.)

27.027 Preparation of Sample—Official

Make ca 200 g sample alk. with lime-H_2O, evap. to dryness, and ash. Ext. crude ash with H_2O contg enough HOAc to decompose carbonates; filter, ignite insol. portion, ext. with HOAc (1+2), and again filter. Insol. portion now contains $CaSiO_3$ and CaF_2, while filtrate contains all H_3BO_3 present.

27.028 Qualitative Test I (*12*)—**Official**

Ash filter contg insol. portion from **27.027**, mix with little pptd SiO_2, transfer to short test tube attached to small U-tube contg few drops H_2O, and add 1–2 ml H_2SO_4. Keep test tube in beaker of H_2O on steam bath 30–40 min. If any F is present, SiF_4 generated is decomposed by H_2O in U-tube and forms gelatinous deposit on walls of tube.

Test filtrate for H_3BO_3 as in **27.010**. If both HF and H_3BO_3 are present, it is probable that they are combined as BF_3. If, however, SiF_4 is de-

tected and H_3BO_3 is not, repeat test without adding the SiO_2, in which case formation of SiO_2 skeleton is conclusive evidence of presence of fluosilicate. In ash contg appreciable quantity of SiO_2, H_2SO_4 liberates SiF_4 rather than HF. Therefore presence of fluosilicate, not fluoride, is indicated.

27.029 Qualitative Test II—Official

Ash filter contg insol. portion from **27.027** in Pt crucible, mix with little pptd SiO_2, and add 1 ml H_2SO_4. Cover crucible with watch glass from underside of which drop of H_2O is suspended, and heat 1 hr at 70–80°, keeping watch glass well cooled. The H_2O decomposes the SiF_4 formed, leaving gelatinous deposit of SiO_2 and etching ring at periphery of drop of H_2O. Test filtrate for H_3BO_3 as in **27.010.**

27.030 Quantitative Method—Official— *See* **24.025–24.031**

FORMALDEHYDE

27.031 Preparation of Sample— First Action

If sample is solid or semi-solid, macerate 100 g with 100 ml H_2O in mortar. Transfer to 800 ml Kjeldahl flask, acidify with H_3PO_4, add 1 ml excess, connect with condenser thru trap, and slowly distill 50 ml. For milk, dil. 100 ml with 100 ml H_2O, and acidify and distill as for solids. With other liquid foods, acidify 200 ml and distill as for solids.

Qualitative Tests—First Action

27.032 *Chromotropic Acid Test (13)*

(a) *Reagent.*—Prep. satd soln of 1,8-dihydroxynaphthalene-3,6-disulfonic acid (ca 500 mg/100 ml) in ca 72% H_2SO_4 (pour 42 ml H_2SO_4 in H_2O, cool, and dil. to 100 ml). Light straw-colored soln should result.

(b) *Test.*—Place 5 ml of the reagent in test tube and add, with mixing, 1 ml distillate, **27.031.** Place in boiling H_2O bath 15 min., and observe during heating period. Presence of HCHO is indicated by appearance of light to deep purple color (depth of color depending on quantity of HCHO present)

27.033 *Hehner-Fulton Test (14)*

(a) *Reagent.—Oxidizing soln.*—To cold H_2SO_4 add, in small portions, equal vol. satd Br-H_2O, cooling thruout operation.

(b) *Test.*—To 6 ml cold H_2SO_4 add slowly and with cooling 5 ml distillate, **27.031.** Place 5 ml mixt. in test tube, and add slowly and with cooling 1 ml aldehyde-free milk, then 0.5 ml of the oxidizing soln. Mix. Purplish-pink color indicates HCHO.

27.034 FORMIC ACID—OFFICIAL— *See* **18.020**

HYDROGEN PEROXIDE

27.035 Qualitative Test *(15)*—**Official**

(Applicable to milk)

(a) *Reagent.*—Dissolve 1 g V_2O_5 in 100 ml H_2SO_4 (6+94).

(b) *Test.*—Add 10–20 drops reagent to ca 10 ml sample and mix. Pink or red color indicates H_2O_2.

MONOCHLOROACETIC ACID *(16)*—OFFICIAL

Qualitative Tests

27.036 *Optical-Crystallographic Properties of Barium Salt*

(Applicable to commercial preservatives)

Dil. 4–5 ml sample to 100 ml, add 6 ml H_2SO_4 (1+1), and ext. with equal vol. ether in separator. If emulsions form, ext. in continuous extractor 1 hr. Transfer ether ext. to separator, add few drops phthln and 5 ml 0.1*N* $Ba(OH)_2$, and shake 30 sec. If aq. layer takes on pink color of phthln, filter thru paper into small beaker. Add ca 0.05*N* HOAc until colorless and evap. to 1–2 ml on steam bath. Let remaining liquid evap. spontaneously in air and finally in desiccator. If 5 ml 0.1*N* $Ba(OH)_2$ does not give pink aq. layer, add 5 ml more before sepg. Repeat extn with $Ba(OH)_2$ soln several times or until pink soln is obtained, evapg each Ba soln in sep. beaker. Examine crystals under polarizing microscope.

Barium monochloroacetate monohydrate crystallizes from H_2O in plates, many of which are hexagonal in habit and frequently form in overlapping layers. Even in material that has been finely powd. for microscopic examination, pointed terminations of the plates, often in pairs, can be observed. In parallel polarized light (crossed nicols) extinction is parallel and sign of elongation is negative on more elongated plates. Plates invariably extinguish sharply with crossed nicols and therefore interference figures are not observed in convergent polarized light (crossed nicols). Since the plates persistently lie in one orientation, significant refractive indices are detd by statistical method, lowest and highest indices respectively being measured on plates showing max. double refraction. These two indices are therefore arbitrarily designated as n_α (min. value) and n_γ (max. value). Two significant refractive indices are: $n_\alpha = 1.582$ and $n_\gamma = 1.611$, both ± 0.002, frequently shown on the platey fragments.

27.037 *Indigo Test*

(a) *Commercial preservatives.*—Dil. 2 ml sample to 100 ml, add 3 ml H_2SO_4, and shake with 100 ml ether. Add 3 ml of the anthranilic acid reagent,

(c), to ether ext., evap. at low temp., filter off any insol. matter, and proceed as in (c), beginning "Test with litmus paper."

(b) *Carbonated beverages, orange juice, and wine.*—Acidify 100 ml sample with 3 ml H_2SO_4 and ext., using either continuous extractor or separator. Add 3 ml of the anthranilic acid reagent, (c), to ether ext. and evap. at low temp. If any insol. matter seps, filter thru small wet paper. To clear liquid in 50 ml beaker add 30 mg Na_2CO_3 and proceed as in (c), beginning "Test with litmus paper."

(c) *Barium monochloroacetate.*—Dissolve 0.17 g Ba salt, **27.036**, in 5 ml H_2O in 10 ml graduate, add 1.05 ml 1.0*N* H_2SO_4, dil. to 10 ml, and mix. Let stand until ppt settles, or filter. Pipet 3 ml clear liquid into small beaker; add 2 ml *anthranilic acid reagent* (1 g +0.3 g NaOH/50 ml) and 30 mg Na_2CO_3 (weighed). Test with litmus paper. If acid, add addnl 30 mg Na_2CO_3. Pour mixt. into test tube and heat in H_2O bath 30 min. Place tube in oven at $125 \pm 5°$ until only moist residue remains. Remove tube from oven, and drop 2 drops NaOH soln (1+1) directly upon residue. (If residue is entirely dry, add 1–2 drops H_2O and let stand until absorbed before adding the NaOH soln.)

Return to oven until completely dry (at least 1 hr); then remove from oven and heat test tube at 310–320° until contents become orange. (This requires 15 sec. to 2 min., but must be carefully watched and tube removed from heat as soon as reaction is complete.) Cool slightly; add 5–7 ml H_2O from wash bottle, splashing the H_2O to incorporate air into it. Warm over flame and blow air thru soln 1–2 min., using pipet or glass tube. Heat to boiling over flame and again blow air thru soln. (As oxidation progresses, soln turns red if monochloroacetic acid is present, then green or blue or combination of two, and finally solid particles of indigo separate out. These tend to rise to surface at first.) Let mixt. stand ca 10 min.; then acidify slightly with HCl (1+1). Let stand 30 min. more, filter, and wash pptd indigo with H_2O to remove acid. Let paper dry in air and preserve as exhibit.

Note: For fusion at 310–320° use brass block having one well to contain test tube and second well to contain thermometer. Block is wrapped with coil of nichrome wire and heat is controlled by variable voltage transformer. Muffle furnaces, microburners, Wood's metal, solder baths, etc., may be used for fusion with equal success.

27.038 *Pyridine Test*

(a) *Commercial preservatives.*—Ext. 2 ml sample as in **27.037**(a). Transfer ether ext. to separator and add small piece of universal indicator paper and enough satd $NaHCO_3$ soln (ca 5 ml) to make aq. layer alk. (pH 7–8) after vigorous shaking. Add enough H_2O to make total vol. of aq. layer ca 10 ml, and shake again. Drain aq. layer into small separator, wash ether with two 5 ml portions H_2O, and add washings to original aq. layer. Wash combined aq. exts once with 5–10 ml ether and discard ether; then add ca 1 ml H_2SO_4 (1+1) in excess of quantity required to neutralize alk. soln (ca 1.5 ml), and ext. acidified soln with two 25 ml portions ether. Wash combined ether exts once with 1 or 2 ml H_2O and let ether soln stand few min. after draining most of H_2O and swirling to get as complete sepn of H_2O from ether as possible. Pour ether thru folded paper into 200 ml flask, and wash separator and paper with two 10 ml portions ether.

To ether filtrate add 0.5 ml pyridine and small glass beads, mix, and evap. ether on steam bath to 2–3 ml. Transfer immediately with eye dropper to 15 ml centrifuge tube, and wash flask successively with 2, 1, and 1 ml portions ether. Evap. liquid in tube to ca 0.3 ml, add enough pyridine to increase vol. to ca 0.5 ml, and place in constant temp. bath at $60 \pm 2°$.

If crystals appear, test is positive. If they do not appear, remove tube from bath and evap. excess pyridine under reduced pressure. (Placing tube in beaker of hot H_2O hastens evapn.) When all liquid has been removed, add 0.5 ml pyridine, mix well, centrifuge, and decant supernatant. Add ca 5 ml ether to residue, shake well, centrifuge, and decant. To residue add 1–3 ml absolute alcohol, varying quantity of alcohol with quantity of ppt, place tube in holder, and heat in hot H_2O or steam bath until ppt dissolves, being careful to swirl tube gently to avoid superheating and to boil alcohol so slowly that no loss occurs. Cool in ice bath, add ca 10 ml ether, mix well, and let stand in ice bath ca 5 min. Centrifuge, pour off supernatant, and wash ppt once with ca 5 ml ether. If tube now contains crystals of pyridine betaine, test is positive.

(b) *Carbonated beverages, orange juice, and wine.*—Acidify 100 ml sample with 3 ml H_2SO_4 and ext., using either continuous extractor or separator. Continue as in (a), beginning "Transfer ether ext. . . . "

Quantitative Method

(Applicable to carbonated beverages, fruit juices, and wine)

27.039 Apparatus

Continuous extractor similar to Fig. 63, **32.059.**—Outer part is made from 43 mm tubing, 45 cm long, with side tube, 25 cm above bottom, fitted with drip tip, ₮ 24/40 joint. Inner tube is made from 12 mm tubing 40 cm long. Receiver is 250 ml conical flask with ₮ neck to fit side tube.

27.040 Reagents

(a) *Silver nitrate soln.*—(1 ml = ca 5 mg $CH_2ClCOOH$.) Dissolve 9 g $AgNO_3$ in H_2O and dil. to 1 L.

(b) *Ammonium thiocyanate soln.*—(1 ml = ca 5 mg $CH_2ClCOOH$.) Dissolve 4.03 g NH_4CNS in H_2O and dil. to 1 L. Stdze against pure NaCl soln, 3.093 g/L, which contains 1.876 g Cl (equiv. to 5 g monochloroacetic acid)/L.

(c) *Ferric indicator.*—Satd soln of $FeNH_4(SO_4)_2 .12H_2O$.

27.041 DETERMINATION

In outer part of continuous extractor place quantity of sample (not >150 ml) contg 50–100 mg $CH_2ClCOOH$. (With commercial preservatives make preliminary diln to permit convenient measurement of proper size aliquot.) Dil. if necessary to 150 ml, add 3–5 ml H_2SO_4, mix, and ext. with ether 2–3 hr. (Extn time for particular app. should be established by detg time required to ext. at least 95% of known quantity of $CH_2ClCOOH$.)

Tilt extractor so as to drain as much ether as possible into flask. Disconnect flask, add 25 ml 1*N* NaOH in excess of that required to make aq. layer alk. to litmus paper after shaking, shake, and evap. ether on steam bath to ca 25 ml, hastening process by passing current of air into mouth of flask. Digest on steam bath 2 hr or boil under reflux condenser 30 min.

Add 50 ml H_2O, 15 ml HNO_3, and known vol. of the $AgNO_3$ soln in excess. Shake 0.5–1 min., add the ferric indicator, and titr. excess Ag with the NH_4CNS soln. In titrn, add the NH_4CNS soln carefully until pink color formed fades slowly on mixing. At this point shake soln ca 30 sec. and filter thru folded paper into second flask. When first flask is empty, wash down walls with ca 50 ml H_2O and add this to filter after all soln has gone thru. When wash H_2O has passed thru, complete titrn. Similarly titr. quantity of $AgNO_3$ soln equal to that added to sample. Difference between 2 titrns is measure of $CH_2ClCOOH$.

Instead of using continuous extractor, $CH_2ClCOOH$ may be extd equally efficiently (except with orange juice) as follows: To 100 ml sample add 3 ml H_2SO_4 and shake in separator with three 100 ml portions ether. Unite ether exts and wash by shaking with two 30 ml portions 1*N* NaOH. Unite the two NaOH solns and digest as above.

5-NITRO-2-PROPOXYANILINE (P-4000)

Qualitative Tests

27.042 *Organoleptic Test (17)—First Action*

Make alk. (pH 7.5–8.0) with 10% NaOH 200 ml liquid food or aq. ext. of 200 g solid food or semi-solid product, **27.068(c)**, and ext. with three 25 ml portions petr. ether. Wash combined exts once with 5 ml H_2O, transfer petr. ether to small beaker or empty dish, let evap. spontaneously, and taste residue. Presence of 5 mg P-4000/L or kg original material may be detected by intensely sweet taste; or 12.5 mg/L or kg original material may be detected by its strong anesthetic effect.

27.043 *Diamine Test (18)—Official*

Pipet 50 ml sample or aq. ext., **27.068(c)**, into separator, make alk. with 10% NaOH, and ext. ca 1 min. with 50 ml petr. ether. Repeat extn with two 50 ml portions petr. ether. Combine exts, wash with 10 ml H_2O, and discard H_2O. Transfer ext. to small beaker and add 4 ml HCl (1+1). Evap. petr. ether on steam bath. Add small piece mossy Sn and keep 5 min. longer on steam bath. Decant soln into test tube; add, dropwise, satd Br-H_2O. Rose-red to deep burgundy-red color is formed if P-4000 is present; this color is destroyed by excess Br.

Quantitative Method (*13*)—Official

(Applicable to non-alcoholic beverages)

27.044 REAGENT

1-Naphthol soln.—Dissolve 50 mg 1-naphthol in 500 ml 1% Na_2CO_3 soln. Prep. fresh daily and store in brown glass bottle.

27.045 DETERMINATION

Pipet 20 ml sample into 250 ml vol. flask, dil. to vol. with H_2O, and mix. Transfer 25 ml aliquot to small separator, and add 10% NaOH soln, dropwise, until just alk. Ext. with three 25 ml portions petr. ether, shaking 1–2 min. each time. Combine exts, wash with 5 ml H_2O, and discard wash H_2O. Transfer ext. to small beaker, and add ca 10 ml H_2O and 0.5 ml 0.1*N* HCl. Evap. petr. ether on steam bath. Remove when few ml petr. ether remain, and let remaining petr. ether evap. spontaneously. Cool soln to ca 20°, add 1.0 ml 0.125% $NaNO_2$ soln, and stir. After 1 min. add 5.0 ml 1-naphthol soln, mix, and dil. to 25 ml. Det. absorbance at 515 mμ within 1 hr against blank carried thru detn. (mg P-4000/25 ml, from std curve) $\times 500$ = mg/L.

27.046 PREPARATION OF STANDARD CURVE

Dissolve 100 mg 5-nitro-2-propoxyaniline in ca 150 ml 60% alcohol. Transfer to 250 ml vol. flask, dil. to vol. with 60% alcohol, and mix. Transfer 1.00, 2.00, 3.00, and 4.00 ml aliquots to 250 ml vol. flasks, dil. to vol. with H_2O, and mix. Using 25 ml aliquots from each flask, proceed as above, beginning "Ext. with three 25 ml portions petr. ether, . . ." Plot absorbance against concn (0 to 0.16 mg/25 ml) final soln.

27.047 PROPIONATES (MOLD INHIBITORS)—FIRST ACTION

—*See* 13.080–13.084

QUATERNARY AMMONIUM COMPOUNDS (QAC)

Qualitative Tests

27.048 *Bromophenol Blue Method (19)—First Action*

(Applicable to milk. Note precautions of **27.056–27.061.**)

Pipet 25 ml milk into 250 ml vol. flask contg 10 mg bromophenol blue, **27.057(d)**, and agitate until solid reagent dissolves. Gradually add 50 ml acetone with shaking; then add, dropwise, enough HCl (1+1) to produce bright yellow color in mixt. (ca 1 ml); then add 0.2–0.3 ml excess. Gradually, with continuous mixing, dil. to mark with acetone. Mix, let stand 30 min., and filter thru folded filter.

Measure 200 ml filtrate in graduated cylinder and pour into 500 ml separator; fill cylinder to 200 ml with H_2O and add to separator. Wash aq. acetone mixt. by shaking with three 50 ml portions petr. ether. When sepd, pour each portion of petr. ether thru filter paper and reserve paper for filtration of ethylene chloride (1,2-dichloroethane) ext. later. Evap. aq. acetone soln on steam bath under current of air until vol. is reduced to 100 ml or less and odor of acetone is gone. Cool, transfer to 250 ml separator with H_2O (reserve beaker), and add 5 ml HCl (1+1).

Pipet 50 ml *ethylene chloride* into separator and shake 1–2 min. Drain lower layer into beaker used for evapn in such manner as to wash down sides and return this liquid to separator, washing beaker with little H_2O. Again shake 2–3 min., let stand until clear, and drain lower layer thru the paper reserved above into 125 ml separator contg 10 ml 1% Na_2CO_3 soln. Stopper, invert separator, and shake carefully 2–3 min., using rotary motion. Reverse funnel to normal position and let stand to sep. Top layer will be usual purple color of alk. soln of strong bromophenol blue; blue color in lower layer is positive test for QAC. To better observe color, drain lower layer into g-s. flask contg 1–2 g anhyd. granular (not powd.) Na_2SO_4 which will absorb on contact any drops of the purple soln which may unavoidably enter flask. Decant ethylene chloride layer into another vessel, if necessary, to avoid any color reflected from colored salt in flask. Ethylene chloride layer must not be filtered, since most papers contain enough residual acid to change blue color of the bromophenol blue-QAC complex to practically invisible yellow.

27.049 *Optical-Crystallographic Properties of the Reineckates (20)—Procedure*

Use reineckate salt obtained in **27.055** or proceed as follows: Add excess of NH_4 reineckate to aq. soln of QAC and stir. In most cases, if >20 mg QAC is present, ppt forms at once. With smaller quantities, let soln stand at room temp. at least 30 min. and then stir 1–2 min. Let mixt. stand several hr, filter thru fine porosity fritted glass crucible, and wash several times with H_2O. Dry ppt with suction, dissolve thru filter with acetone, and evap. off the acetone. Dissolve dry residue by warming with min. quantity alcohol. If considerable quantity of ppt is used, crystals deposit on cooling. Filter thru fritted glass crucible and dry by suction. With <30 mg ppt, dissolve in 10 ml alcohol and let solvent evap. on warm, but not hot, surface with aid of gentle current of air.

Det. optical-crystallographic properties of the crystals as in **32.236** and compare with those listed in **27.050** or with those detd on crystals obtained from known QAC compound.

27.050 *Table of Optical-Crystallographic Properties of the Reineckates*— *See* page 393

Quantitative Methods

Ferricyanide Method (19)—Official

(Applicable to commercial preservatives)

27.051 REAGENTS

(**a**) *Acetate buffer soln.*—Dissolve 130 g NaOAc $.3H_2O$ in H_2O, add 42 ml HOAc, and dil. to 500 ml.

(**b**) *Ferricyanide soln.*—Dissolve 6.6 g $K_3Fe(CN)_6$ in H_2O and dil. to 1 L.

(**c**) *Zinc sulfate soln.*—Dissolve 20 g $ZnSO_4$ $.7H_2O$ in 180 ml H_2O.

(**d**) *Thiosulfate soln.*—Dissolve 5 g $Na_2S_2O_3$ $.5H_2O$ in H_2O, dil. to 1 L, and stdze as in **42.036.** (1 ml 0.02N soln = 0.02142 g alkyldimethylbenzylammonium chloride, molecular wt 357.)

27.052 DETERMINATION

Det. approx. quaternary ammonium salt concn as follows: Pipet 1 ml buffer soln, 2 ml $K_3Fe(CN)_6$ soln, and 20 ml H_2O into each of 4 small erlenmeyers. To these flasks add 0.5, 1.0, 2.0, and 4.0 ml, resp., of sample, mix, and filter. Add 2 ml more of sample to each filtrate, mix, and compare results with table, **27.053.**

Into 100 ml Kohlrausch flask pipet aliquot of sample contg ca 0.5 g quaternary ammonium salt, as indicated by **27.053,** dil. if necessary to 50 ml, add 5 ml buffer soln, and mix. Then add from pipet 30 ml $K_3Fe(CN)_6$ soln while swirling flask. Dil. to 100 ml mark with H_2O and mix. After 30 min., filter, discarding first 10–15 ml filtrate. Pipet 50 ml filtrate into 500 ml erlenmeyer, and add 100 ml H_2O and 1–2 g KI. Rotate flask until salt dissolves, add 10 ml HCl (1+1), mix, and let stand 2 min. Add 10 ml $ZnSO_4$ soln, mix, and titr.

27.050 *Optical-crystallographic properties of reineckates of quaternary ammonium compounds*

COMPOUND	QUANTITATIVE FACTOR	REFRACTIVE INDICES[a]			OPTIC SIGN	EXTINCTION[b]	ELONGATION	HABIT
		α	β	γ				
Cetyldimethylbenzylammonium Reineckate (Zettyn ®)	0.5384	1.572	1.651	1.660	—	i, s		Rhomboid plates
Alkyldimethylbenzylammonium Reineckate	0.5582	1.576		1.651	—	s		Rhomboid plates
Lauryldimethylbenzylammonium Reineckate (DC-12)	0.5458	1.576	1.669^i	1.678	—	p, s	—	Rhomboid plates
Di-isobutylphenoxyethoxyethyldimethylbenzylammonium Reineckate (Hyamine 1622 ®, Phemerol ®)	0.6130	1.577	1.671	1.678	—	p, i	+	Rods, plates, Fibrous
Cetylpyridinium Reineckate (Ceepryn ®)	0.5458	Unsatisfactory for optical study				p	—	Fibrous
Lauryldimethyldichlorobenzylammonium Reineckate (Dichloran)	0.5220	1.582	1.593^i	1.677	—	p	—	Rods and plates
Di-isobutylcresoxyethoxyethyldimethylbenzylammonium Reineckate (Hyamine 10-X ®)	0.6028	1.582	1.638	1.670	—	p	+	Plates
Dodecylacetamidodimethylbenzylammonium Reineckate (Dobenzyl chloride)	0.5911	1.582		1.664		p	—	Plates and rods
Cetyldimethylethylammonium Reineckate (Ethyl Cetab)	0.6135	1.587	1.599	1.626		p	+	Plates and rods
Cetyltrimethylammonium Reineckate (Cetab)	0.6045	1.591	1.609^i	1.616		p	+	Rods
Triethylbenzylammonium Reineckate	0.4460	1.593	1.687	1.697 (ca)	—	s		Rhomboid plates
Laurylpyridinium Reineckate	0.5112	1.609	1.636^i	1.651		p	+	Plates

[a] Refractive indices ±0.003, at 24–26°; i = intermediate index.
[b] Extinction: p = parallel; i = inclined; s = symmetrical.

with the $Na_2S_2O_3$ soln, adding starch indicator, **4.004(f)**, when color fades to tinge of yellow. Make blank detn including all of above operations but substituting H_2O for sample. Calc. QAC content from difference in 2 titrns.

If sample contains ca 0.5 g QAC/100 ml, instead of proceeding as above pipet 100 ml sample into 200 ml vol. flask, add 10 ml buffer soln and 30 ml $K_3Fe(CN)_6$ soln, dil. to vol. with H_2O, mix, let stand 30 min., filter, and titr. 100 or 150 ml aliquot filtrate as above.

27.053 *Approximation of content of alkyldimethylbenzylammonium chloride (mol. wt 357)*

QUATERNARY AMMONIUM CHLORIDE PER CENT	SAMPLE ADDED			
	A 0.5 ML	B 1.0 ML	C 2.0 ML	D 4.0 ML
8.4 or more	No ppt	No ppt	No ppt	No ppt
5	Ppt	No ppt	No ppt	No ppt
2.5	Ppt	Ppt	No ppt	No ppt
1.25	Ppt	Ppt	Ppt	No ppt
1 or less	Ppt	Ppt	Ppt	Ppt

Reineckate Method (21)—Official

(Applicable to preservatives, tinctures, and isotonic solns)

27.054 REAGENT

Reineckate reagent.—Place 0.75 g NH_4 reineckate ($NH_4[Cr(NH_3)_2(SCN)_4].H_2O$; mol. wt =354.47) in 125 ml erlenmeyer, add 50 ml H_2O, stopper, shake ca 2 min., and filter.

27.055 DETERMINATION

Place 100 ml sample contg 10–100 mg QAC in 250 ml beaker; add, with stirring, 5 ml portions reineckate reagent until liquid is bright pink. Let stand 30 min. and add more reagent unless supernatant is deep pink. Stir again 1–2 min. After several hr filter thru fine porosity fritted glass crucible, and wash beaker and filter with at least three 15 ml portions H_2O. (It is unnecessary to transfer all ppt to crucible.) Wash down sides of crucible with H_2O and dry by suction. If ppt forms cake in filter, mix with the wash H_2O with stirring rod used before.

Dissolve reineckate salt in acetone as follows: Set up suction app. to fit glass crucible, using as receiver side-arm test tube for application of suction. With 5 ml pipet, wash down sides of beaker used for pptn and add this liquid to crucible. Rinse beaker second time and add to liquid in crucible. Stir to dissolve and draw liquid thru with suction. Wash out beaker third time and wash down sides of crucible several times with small portions of acetone. When liquid passing thru is colorless, disconnect, and wash into test tube with acetone any pink material which may have dried on bottom or outside of crucible or on inside of funnel. Discard small quantity of greenish solid in crucible due to impurities and decomposition products of reagent.

Transfer acetone soln to tared beaker (50 ml beaker for 20 mg or less QAC and 100 ml beaker for >20 mg) and evap. on warm (but not hot) surface. If few drops of moisture remain, pass gentle current of air into beaker until it appears dry. Dissolve residue by warming in 10 ml alcohol (or more, if needed); let solvent evap. spontaneously, dry in desiccator, and weigh. Wt QAC =factor (**27.050**) × wt ppt. Ppt may be used for detn of optical-crystallographic properties, **27.050**.

(To remove the greenish solid from crucible, add 10–12 ml HCl (1+1) and stir to dissolve. Draw liquid thru by suction and wash several times with H_2O. Reverse crucible and wash by filling bottom cavity with solvent. Use 2 fillings each of H_2O, alcohol, H_2O, acetone, and H_2O in order given.)

Bromophenol Blue Method (19)*

(PRECAUTION: Have all glassware scrupulously clean, and especially avoid soap, since reaction occurs between soap and QAC. If soap is used in cleansing, rinse all glassware with H_2O, and as extra precaution rinse all pipets with alcohol and dry by suction.)

27.056 APPARATUS

Steam distillation apparatus.—*See* Fig. 31, **18.015(a)** for generator. Use 500 ml or 1 L distn flask, fitted with spray tube, **8.025(c)**, reaching to within 1 or 2 cm of bottom of flask (all connections ₮ joints), and with stopper for steam inlet (to be used during early part of distn). Suitable app. is described in **19.105**.

27.057 REAGENTS

(**a**) *Sodium carbonate soln.*—Dissolve 5 g Na_2CO_3 in 500 ml H_2O.

(**b**) *Sodium sulfate.*—Anhyd. granular (not powd.). (Mallinckrodt A.R. granular grade is satisfactory.)

(**c**) *D.C. 12.*—Lauryldimethylbenzylammonium chloride, or other solid quaternary ammonium compound.

(**d**) *Bromophenol blue soln.*—Dissolve 40 mg tetrabromophenolsulfonphthalein in warm H_2O, cool, and dil. to 100 ml.

The bromophenol blue should pass following test for purity: Place 20 mg bromophenol blue in 125 ml separator; add 50 ml ethylene chloride and 5 ml 1% Na_2CO_3 soln, and shake until dissolved. Let stand until mixt. seps into 2 layers. Lower layer should be colorless, upper layer purple. Add 10 ml soln contg 0.1 mg D.C. 12 or

* Official for bottled beverages contg fruit juices, beer, and table sirup; first action for eggs.

other QAC, shake again, and let sep. Lower layer should be clear blue. Drain lower layer and examine in spectrophotometer. Absorption peak should be at ca 608 mμ. Compare absorption curve with that of sample purified as in **27.058.** If test gives yellow or green color or if absorption curve is essentially different from that of purified sample, purify as in **27.058.**

27.058 PURIFICATION OF BROMOPHENOL BLUE

Place 2 g bromophenol blue in 400 ml beaker and dissolve in 25 ml 1% Na_2CO_3 soln. Transfer to 1 L separator, using ca 300 ml H_2O. Add 500 ml ethylene chloride and shake. Add 1 ml soln contg 10 mg D.C. 12 or other QAC and shake until thoroly extd. If lower layer is yellow, repeat addn of D.C. 12 soln in 1 ml portions with shaking until upon sepn of the 2 layers, lower one has greenish tint. Drain lower layer and discard. Add 200 ml ethylene chloride and 1 ml D.C. 12 soln to separator and shake. This time lower layer should be clear blue. If layer is green, drain and repeat addn of ethylene chloride and D.C. 12 until blue soln is obtained. Wash aq. layer with 100 ml portions ethylene chloride until lower layer is colorless or only faint blue. Acidify aq. layer with HCl and ext. yellow ppt with ethylene chloride until aq. soln is only faint yellow. Distill off most of the ethylene chloride and permit remainder to evap. spontaneously in beaker. Grind residual powder. Test portion for purity as in **27.057(d)** and if suitable, use as reagent.

27.059 PREPARATION OF STANDARD CURVE

Stdze 1% soln QAC to be detd as in **27.052.** (If this compound is not available, use any solid QAC of known composition such as D.C. 12, lauryldimethylbenzylammonium chloride. If necessary, prep. std soln from commercial soln stdzd by ferricyanide method, **27.052.**) By carrying out method below, ascertain max. and min. concns of this compound that produce, in 50 ml ethylene chloride, absorbance at 610 mμ suited to color-measuring instrument used. Then prep. set of 3 or more stds contg, in 50 ml, quantities of the QAC covering range between these max. and min. concns, and plot curve as directed below. (If neutral wedge photometer or Beckman spectrophotometer is used, 0.0, 0.1, 0.2, and 0.25 mg/50 ml are suitable stds.)

Pipet 50 ml of each std into separator; add 3 ml of the bromophenol blue soln, 1 ml HCl (1+1), and 50 ml ethylene chloride; and shake 2–3 min. When clear, drain lower layer into another separator contg 10 ml of the Na_2CO_3 soln, and shake 2–3 min. Let stand until clear, drain lower layer into g-s. flask contg 1–2 g of the Na_2SO_4, and after 30 min. read in instrument. (Use same or similar cell for all stds, and light filter centering at 610 mμ; 1″ cell is satisfactory with neutral wedge photometer.) Plot scale readings, if these are in terms of absorbance or proportional to it, against concns used; if instrument reads in terms of transmittance, convert readings to absorbance before plotting.

27.060 PREPARATION OF SAMPLE

(a) *Bottled beverages containing fruit juices.*—Mix thoroly, and measure 50 ml sample into graduated cylinder. Filter on 7 cm büchner and dil. filtrate to 100 ml with H_2O (Soln *A*). Place filter paper in 400 ml beaker and ext. with small portions of alcohol until no more color is extd and paper remains white. Transfer alc. ext. to 500 ml distg flask; add 10 mg bromophenol blue, 2 ml HCl (1+1), and 100 ml H_2O. Steam distill and collect vol. distillate at least 100 ml greater than vol. alcohol in ext. Cool residue in distg flask, transfer to separator, wash with 40, 30, and 30 ml portions petr. ether, and proceed as in **27.061.**

Also take suitable aliquot of Soln *A* (first try 5 ml), transfer to separator, add 3 ml of the bromophenol blue soln and 1 ml HCl (1+1), and proceed as in **27.061.**

(b) *Beer.*—Place 100 ml decarbonated beer, **10.001,** in steam distn flask and add 10 mg bromophenol blue and 2 ml HCl (1+1). Steam distill and collect ca 200 ml distillate. Cool residue, transfer to separator, wash with 100 and 50 ml portions petr. ether, and proceed as in **27.061.**

(c) *Table sirup.*—Transfer 20 g sample to 100 ml vol. flask, dil. to vol. with H_2O, and mix thoroly. Pipet aliquot of soln into separator, add 5 ml of the bromophenol blue soln and 1 ml HCl (1+1), and proceed as in **27.061.**

(d) *Eggs.*—Weigh 12.5±0.25 g well-mixed sample in tared 50 ml beaker. Add 10 ml H_2O, mix well with rod, pour carefully into 250 ml vol. flask, and wash beaker with 5–10 ml more H_2O, adding washings to flask. While swirling flask, gradually add acetone, little at time, mixing constantly, until flask is filled to mark; stopper and invert several times. Let stand 10–15 min. and filter thru folded paper (Whatman No. 12, 18.5 cm) into 250 ml graduated cylinder until 200 ml filtrate is obtained. Pour filtrate into 1 L separator, wash down sides of cylinder with 25 ml acetone, and add to separator; fill cylinder to 250 ml with H_2O and add to separator. Add 25 ml HCl (1+1) to separator and mix. Ext. liquid in separator with petr. ether, using 300, 250, 150, and 100 ml, and shaking gently to prevent formation of emulsions. Transfer extd aq. layer to 600–800 ml beaker, add 2–3 glass beads, and evap. to 50–75 ml on steam bath.

After evapn, add 10 mg bromophenol blue and

wash down sides of beaker with little H_2O. When soln is cool, pipet 50 ml ethylene chloride into beaker, letting solvent flow down sides of beaker. Pour contents of beaker into 250 ml separator, washing out beaker with little H_2O. Shake ca 1 min. Return liquid to beaker, letting it flow down sides of beaker. Again return liquid to separator and shake ca 2 min. Proceed as in **27.061**, beginning "Let stand until clear . . . "

27.061 DETERMINATION

Pipet 50 ml ethylene chloride into separator, **27.060**(**a**), (**b**), or (**c**), and shake 3–4 min. Let stand until clear, drain lower layer into second separator contg 10 ml of the Na_2CO_3 soln, and shake 3–4 min. Let sep. and observe lower layer. If blue, quaternary base is present. Judge from depth of color whether or not it is suitable for reading in photometer. If color is suitable for reading without diln, drain lower layer into g-s. flask contg 1–2 g of the Na_2SO_4, let stand 30 min., transfer to suitable cell, and read color in instrument at 610 mμ. Det. quantity QAC present from std curve, **27.059**, and calc. to mg/100 ml.

If color is too deep for direct reading, acidify contents of second separator with 1 or 2 ml HCl (1+1), shake until contents become yellow, and return to first separator. Pipet second 50 ml portion ethylene chloride into first separator, shake 3–4 min., let stand until lower layer is clear, and drain lower layer into flask. (If sample is known to contain >1 mg QAC/100 ml, entire 100 ml ethylene chloride may be added at one time.)

Det. proper aliquot as follows: Pipet 5 ml into 125 ml separator, dil. with 25 ml ethylene chloride, add 10 ml 1% Na_2CO_3 soln, and carefully shake 2 min. Let sep. and observe lower layer. If depth of color is suitable for reading, dil. to 50 ml by adding 20 ml ethylene chloride from pipet, shake 1 min., let settle, drain lower layer, dry, and read. If color is not deep enough, add more of the soln in 5 ml increments until suitable color is obtained, add solvent if necessary to total vol. of 50 ml, shake, drain, dry, and read in instrument.

When proper aliquot has been detd, check as follows: Pipet aliquot of ethylene chloride soln into 50 ml vol. flask, fill to mark with ethylene chloride, and pour into 125 ml separator. Pipet 10 ml 1% Na_2CO_3 soln into vol. flask, swirl, pour into separator, and wash out vol. flask with 2–3 ml H_2O from wash bottle. Shake, settle, drain, dry, and read, adding ca 5 mg dry bromophenol blue to separator if aliquot used was 10 ml or less.

For bottled beverages contg fruit juices, add quantity of QAC found in residue to quantity found in Soln *A* to obtain total quantity in sample.

Eosin Yellowish Method (22)—First Action

(Applicable to aq. solns and milk)

27.062 APPARATUS

(**a**) *Centrifuge.*—Clinical high speed type fitted for 50 ml tubes. International No. 2 centrifuge with head No. 241 at speed of 2500 rpm is also satisfactory.

(**b**) *Centrifuge tubes.*—Heavy wall, 40 ml centrifuge tubes, Pyrex, No. 8400 or equiv.

(**c**) *Test tubes.*—Pyrex, g-s., 15×150 mm.

27.063 REAGENTS

(**a**) *Acetylene tetrachloride.*—Should give distinct pink color in lower layer after sepn, when 5 ml is shaken for 1 min. with 2 ml buffer soln (**e**), 0.5 ml eosin yellowish soln (**c**), and 5 ml aq. soln contg 1 ppm Cetab, Dobenzyl chloride, Et Cetab, Hyamine 10-X, or laurylpyridinium chloride, or 2 ppm lauryldimethylbenzylammonium chloride. If reagent does not meet this test, distill under reduced pressure, rejecting first 10% of distillate and collecting ca 80% of vol. placed in distn flask.

(**b**) *Lactic acid soln.*—50%. Add 41 g H_2O to 59 g lactic acid, 85% reagent grade, and mix.

(**c**) *Eosin yellowish soln.*—Dissolve 25 mg D&C Red No. 22 in H_2O and dil. to 50 ml.

(**d**) *Sodium hydroxide soln.*—4*M*. Dissolve 32 g NaOH in H_2O and dil. to 200 ml.

(**e**) *Citrate buffer soln.*—pH 4.5. Dissolve 25 g citric acid in 75 ml H_2O and add enough 50% NaOH soln (ca 13 ml) to bring pH to 4.5.

(**f**) *Aerosol OT stock soln.*—Prep. soln of dioctyl Na sulfosuccinate to contain 100 mg/100 ml. Det. strength as follows: Pipet 2 ml soln contg, in 100 ml, 100 mg of the QAC to be detd, into g-s. test tube contg 2 ml acetylene tetrachloride, 2 ml buffer soln, and 0.5 ml eosin yellowish soln. Carefully add the Aerosol OT soln from buret in small amounts, shaking mixt. violently for at least 30 sec. after each addn until, after sepn into 2 layers, only a light pink is noticeable when test tube is placed against white background. Continue addns in 0.01 or 0.02 ml portions until lower layer is no longer pink.

(**g**) *Aerosol OT std soln.*—Dil. to 100 ml such quantity of the stock soln (**f**) as will produce soln 1 ml of which is equiv. to 0.1 mg of the QAC to be detd. Stdze against std soln (1 ml = 0.1 mg) of QAC to be detd.

27.064 DETERMINATION

(**a**) *Milk.*—Pipet 15 ml acetylene tetrachloride into centrifuge tube, add 6 ml lactic acid soln and 15 ml milk to be tested, stopper, and shake ca 3 min. Add 6 ml NaOH soln and mix carefully until curd seps thruout mixt.; then shake at least 30 sec. Centrifuge at high speed (ca 3200

rpm) 7 min. Decant serum and discard; puncture layer of curd at 2 points and drain acetylene chloride ext. into small beaker. Avoiding any drops of the aq. soln, transfer with pipet 5 ml of the ext. into g-s. test tube contg 2 ml buffer soln and 0.5 ml of the eosin yellowish soln; stopper and shake ca 2 min. Let stand to sep. and observe color of lower layer. If color is faint, place against white background. If layer is pink, QAC is present. If deep pink or red, titr. with the std Aerosol OT soln; after each addn of the std soln, shake mixt. violently 0.5 to 1 min., let sep., and observe lower layer. Continue addns until no pink is observed in lower layer when placed against white background or compared with blank detn. Titrn found represents quantity QAC in 5 ml sample. Calc. to ppm.

(**b**) *Water solns.*—Pipet 5 ml sample into g-s. test tube contg 2 ml acetylene tetrachloride, 2 ml buffer soln, and 0.5 ml eosin yellowish soln, and proceed as in (**a**).

SACCHARIN

Qualitative Tests

27.065 *Organoleptic Test—Official*

Acidify with HCl 50 ml non-alc. liquid food or aq. ext. of 50 g solid or semi-solid product, **27.068**(**c**), and ext. with three 25 ml portions ether. Wash combined ether exts once with 5 ml H_2O, transfer to small beaker or evapg dish, let ether evap. spontaneously, and taste residue. (Presence of 20 mg saccharin/L or kg of original sample can usually be detected by its sweet taste.) Confirm by heating with NaOH and detecting salicylic acid formed thereby as in **27.066.**

27.066 *By Conversion to Salicylic Acid—Official*

Acidify with HCl 50 ml non-alc. liquid food, or equiv. quantity of aq. ext., **27.068,** and ext. with 3 portions of ether as in **27.065.** Dissolve residue remaining after evapn of ether in little hot H_2O and test small portion of soln for salicylic acid as in **27.073** or **27.074.**

Dil. remainder of soln to ca 10 ml and add 2 ml H_2SO_4 (1+3). Heat to boiling and add slight excess of 5% $KMnO_4$ soln dropwise; partly cool soln, dissolve ca 1 g NaOH in it, and filter mixt. into Ag dish (Ag crucible lids are suitable). Evap. to dryness and heat 20 min. at 210–215°. Dissolve residue in H_2O, acidify with HCl, and test ether ext. for salicylic acid as in **27.073** or **27.074.** By this method all so-called "false saccharin" (*23*) and any salicylic acid naturally present (also added salicylic acid when not present in too large a quantity) are destroyed, whereas 5 mg saccharin/L is detected with certainty.

27.067 *Phenol-Sulfuric Acid Test* (*24*)—*Official*

(Applicable to non-alc. beverages, semi-solid prepns, and baked goods)

Prep. ether ext. of sample as follows:

(**a**) *Non-alcoholic beverages.*—Add 3 ml HCl to 25 ml sample in separator. If vanillin is present, remove by extg with several portions of petr. ether. Discard petr. ether. Ext. with 50, 25, and 25 ml ether-petr. ether (1+1). Wash combined ether exts once with 5 ml H_2O, remove major portion of solvent, transfer to 30 ml beaker, and evap. at room temp.

(**b**) *Semi-solid preparations.*—Transfer 25 g sample to 100 ml vol. flask with small quantity of hot H_2O and add enough boiling H_2O to make ca 75 ml. Let mixt. stand 1 hr, shaking occasionally. Then add 3 ml HOAc, mix thoroly, add slight excess (5 ml) of 20% neutral $Pb(OAc)_2$ soln, dil. to mark with cold H_2O, mix, let stand 20 min., and filter. Transfer 60 ml or more of filtrate to separator and proceed as in (**a**).

(**c**) *Baked goods.*—Grind 25 g sample, mix thoroly with 50 g washed and ignited sea sand, and ext. with petr. ether in Soxhlet app. until ca fat-free (1–2 hr). Transfer extd mass to 300 ml erlenmeyer, add 100 ml alcohol, and reflux on boiling H_2O bath 30 min., shaking frequently. Filter thru büchner contg 7 cm Whatman No. 2 paper wet with alcohol. Transfer alc. filtrate to 100 ml beaker, evap. to ½ vol., add 50 ml H_2O and enough 10% Na_2CO_3 soln to make alk., and evap. to 50 ml. Transfer aq. soln to separator and proceed as in (**a**).

To residue remaining after evapn of solvent add 5 ml *phenol-H_2SO_4 reagent* (pure colorless cryst. phenol dissolved in equal wt H_2SO_4) and heat 2 hr at 135–140°. Cool, dissolve in small quantity of hot H_2O, and pour into ca 250 ml H_2O. Add small quantity of Filter-Cel, let stand 3 hr or overnight, and filter. Make alk. with 10% NaOH soln and dil. to 500 ml. Magenta or reddish-purple color develops if saccharin is present. Yellow, buff, or pale salmon shade is not significant.

Quantitative Methods

General Method I.—Official

27.068 PREPARATION OF SAMPLE

(**a**) *Fruit juices and sirups.*—Transfer 100–200 g sample to 250 ml vol. flask with little H_2O and dil. to ca 200 ml with H_2O. Add 5 ml HOAc and mix. Add slight excess of 20% neutral $Pb(OAc)_2$ soln, mix thoroly, dil. to mark with H_2O, again mix thoroly, and filter.

(**b**) *Alcoholic liquids.*—Heat 100–200 ml liquid on steam bath to remove alcohol (usually done by evapg to ½ original vol.). With heavy sirups, dil.

liquid with equal vol. H_2O before beginning evapn. After removal of alcohol, transfer liquid to 250 ml vol. flask and proceed as in (**a**).

(**c**) *Solid or semi-solid preparations.*—Transfer 50–75 g sample to 250 ml vol. flask with little hot H_2O and add enough boiling H_2O to make ca 200 ml. Let stand 2 hr, shaking occasionally. Add 5 ml HOAc, mix thoroly, add slight excess of 20% neutral $Pb(OAc)_2$ soln, dil. to mark with cold H_2O, mix, let stand 20 min., and filter.

27.069 DETERMINATION

Transfer 150 ml filtrate, **27.068,** to separator, add 15 ml HCl, and ext. with three 80 ml portions ether, shaking separator 2 min. each time. Wash combined ether exts once with 5 ml H_2O, remove ether by distn, and transfer residue to Pt crucible with little ether; or, if substances difficultly sol. in ether are present, use alternately small portions of H_2O, and ether. Evap. ether on steam bath, add to residue 2–3 ml 10% Na_2CO_3 soln (or enough to make mixt. strongly alk.), rotate so that all saccharin is brought in contact with soln, and evap. to dryness on steam bath.

To dry residue add 4 g mixt. of equal parts of anhyd. Na_2CO_3 and K_2CO_3. Heat gently at first and then to complete fusion 30 min. (Fusion may be conducted by closely fitting crucible into hole cut into piece of heavy asbestos board so that ⅓ of crucible projects above asbestos, and heating lower portion of crucible by large Bunsen, Meker, or similar burner.) Cool, dissolve melt in H_2O, add ca 5 ml Br-H_2O, acidify with HCl, filter, wash paper with little H_2O, dil. filtrate and washings to ca 200 ml, heat to boiling, and slowly add excess of $BaCl_2$ soln (ca 10%). Let mixt. stand overnight, collect $BaSO_4$ on filter or on gooch, wash until Cl-free, dry, ignite, cool, and weigh. Correct results thus obtained for any S present in fusion mixt. as found by blank detn. Calc. equiv. quantity of saccharin by multiplying corrected wt $BaSO_4$ by 0.7848.

Instead of the mixed Na and K carbonates, 3–4 g Na_2O_2 may be used for fusion. In this case Ni crucible must be used, and time of fusion may be reduced to 5 min. Sepn of little $PbCl_2$ during extns does not interfere with accuracy of method.

27.070 *General Method II. (By Sublimation) (25)—First Action*

Acidify 200 ml sample with 15 ml HCl and ext. with three 50 ml portions CCl_4. Discard CCl_4, and ext. aq. layer with three 80 ml portions ether. Let ether ext. evap. to small vol. and transfer to sublimator with small quantity of ether or alcohol. Evap. to dryness at room temp. or on H_2O bath, depending on whether ether or alcohol was used to transfer residue. Sublime residue 1 hr at pressure of 1–2 mm and temp. of 140–160°. (Raise temp. so slowly that ca ½ hr is required to reach 140°.) Wash saccharin from condenser bulb of sublimator with warm alcohol into weighed beaker, and repeat sublimation until no further residue appears on condensing bulb. Evap. alcohol on H_2O bath, heat residue 2 hr at 100°, cool, and reweigh beaker.

27.071 *Special Method for Non-Alcoholic Beverages (26)—Official*

Add 2 ml HCl to 50 ml sample in separator. Ext. with two 50 ml portions ether. Filter ether exts thru cotton, and wash combined filtrates with ca 5 ml H_2O contg 1 drop HCl.

Sep. ether layer and evap. to dryness on H_2O bath. Add to residue 5 ml NH_3-free H_2O and 6 ml HCl, and evap. soln to ca 1 ml on hot plate, stirring constantly. Again add 5 ml NH_3-free H_2O and 6 ml HCl, and evap. to ca 1 ml. Dil. to 50 ml with NH_3-free H_2O and dil. 2 ml of this soln to 25 ml with NH_3-free H_2O. Add 1 ml Nessler reagent, **31.007**(**b**), and compare with NH_4Cl stds in usual manner; 0.2921 g NH_4Cl = 1 g saccharin, insol. form ($C_7H_5NO_3S$), and 1.317 g Na salt ($C_7H_4NNaO_3S.2H_2O$). For convenience prep. NH_4Cl std equiv. to 200 ppm insol. form of saccharin.

SALICYLIC ACID

27.072 Preparation of Sample—Official

(**a**) *Non-alcoholic liquids.*—Many liquids may be extd directly as in **27.073** or **27.075** without further treatment. If troublesome emulsions form during extn, pipet 100 ml into 250 ml vol. flask and add ca 5 g NaCl, shaking until dissolved. Dil. to mark with alcohol, shake vigorously, let stand 10 min., shaking occasionally, filter, and treat aliquot of filtrate as in (**b**).

(**b**) *Alcoholic liquids.*—Make 200 ml of sample alk. to litmus paper with ca 10% NaOH soln and evap. on steam bath to ca ⅓ its original vol. Dil. to original vol. with H_2O and filter if necessary.

(**c**) *Solid or semi-solid substances.*—Grind sample and mix thoroly. Transfer convenient quantity (50–200 g according to consistency of sample) to 500 ml vol. flask, add enough H_2O to make ca 400 ml, and shake until mixt. becomes uniform. Add 2–5 g $CaCl_2$ and shake until dissolved. Make distinctly alk. to litmus paper with ca 10% NaOH soln, dil. to mark with H_2O, shake thoroly, let stand at least 2 hr, shaking frequently, and filter.

Qualitative Tests

27.073 *Ferric Chloride Test—Official*

Place 50 ml sample or equiv. quantity of aq. ext., prepd as in **27.072,** in separator; add $\frac{1}{10}$ its vol. HCl (1+3) and ext. with 50 ml ether. If mixt. emulsifies, add 10–15 ml petr. ether (b.p.

$<60°$) and shake. If this treatment fails to break emulsion, centrifuge, or let stand until considerable portion of aq. layer seps; drain latter, shake vigorously, and again let sep. Wash ether layer with two 5 ml portions H_2O, evap. greater portion of ether in porcelain dish on steam bath, let remainder evap. spontaneously, and add 1 drop 0.5% neutral $FeCl_3$ soln. Violet color indicates salicylic acid.

If coloring matter or other interfering substance is present in residue after evapn of ether, purify the salicylic acid by one of following methods:

(**a**) Dissolve original residue from ether ext., obtained as above, in ca 25 ml ether; transfer soln to separator and shake with equal vol. H_2O made distinctly alk. with several drops 10% NH_4OH. Let sep., filter aq. layer thru wet filter into porcelain dish, evap. almost to dryness, and test residue with $FeCl_3$ as above.

(**b**) Dry original residue from ether ext., obtained as above, in desiccator over H_2SO_4 and ext. with several 10 ml portions CS_2 or petr. ether (b.p. $<60°$), rubbing contents of dish with glass rod and filtering successive portions of solvent thru dry paper into second porcelain dish. Evap. greater portion of solvent on steam bath, let remainder evap. spontaneously, and test residue with $FeCl_3$ as above.

(**c**) With few ml of ether, transfer original residue from ether ext. obtained as above to small porcelain crucible, and let solvent evap. spontaneously. Cut hole in asbestos board large enough to admit ca $\frac{2}{3}$ of crucible, cover crucible with small round-bottom flask filled with cold H_2O, and heat over small flame until any salicylic acid present sublimes and condenses upon bottom of flask. Test sublimate with $FeCl_3$ as above.

27.074 *Jorissen Test (27)—Official*

Dissolve residue from ether ext., **27.073**, or, if impurities are present, purified material obtained as in **27.073**(**a**), (**b**), or (**c**), in little hot H_2O. Cool 10 ml soln in test tube and add 4 or 5 drops 10% KNO_2 soln, 4 or 5 drops HOAc (ca 50%), and 1 drop 1% $CuSO_4$ soln. Mix thoroly, boil liquid 0.5 min., and let stand 2 min. In presence of salicylic acid Bordeaux-red color develops.

Quantitative Method—Official

27.075 EXTRACTION

Transfer to separator 100 ml sample, or quantity of soln prepd as in **27.072** that represents not <20 g original material. If alk., neutralize to litmus with HCl (1+3) and add excess of HCl equiv. to 2 ml acid for each 100 ml soln. Ext. 4 times with ether, using for each extn vol. ether equiv. to $\frac{1}{2}$ vol. aq. layer. If emulsion forms on shaking, this may usually be broken by adding little ($\frac{1}{5}$ vol. ether layer) petr. ether (b.p. $<60°$) and shaking again, or by centrifuging. If small quantity of emulsion still persists, let remain with aq. layer, where frequently it is broken during next extn. If emulsion remains after fourth extn, sep. it from clear ether and clear aq. layer and ext. separately with 2 or 3 small portions of ether.

Combine ether exts, wash with vol. H_2O equal to $\frac{1}{10}$ total vol. ether exts, let sep., and discard aq. layer. Wash in this way until aq. layer after sepn yields yellow color upon addn of Me orange soln and 2 drops $0.1N$ NaOH. Slowly distill greater part of ether, transfer remainder to porcelain dish, and let it evap. spontaneously. If no interfering substances are present, proceed as in **27.076**; if interfering substances are present, purify residue by one of following methods:

(**a**) Thoroly dry residue *in vacuo* over H_2SO_4. Ext. 10 times with 10–15 ml portions CS_2 or petr. ether (b.p. $<60°$), rubbing contents of dish with glass rod, and filter successive portions of solvent thru dry filter into porcelain dish. Test extd residue with drop *2% Fe alum soln*, and if it gives reaction for salicylic acid, dissolve in H_2O; acidify soln with HCl (1+3), ext. with ether, evap., ext. dry residue thus obtained with CS_2 or petr. ether, and add to ext. first obtained. Distill greater portion of the CS_2 or petr. ether and let remainder evap. spontaneously. Proceed as in **27.076**.

(**b**) Dissolve residue in 40–50 ml ether. Transfer ether soln to separator and ext. with three 15 ml portions 1% NH_4OH. (If fat is known to be present in original ether ext., ext. latter directly with 4 portions of the NH_4OH instead of 3.) Combine alk. aq. exts, acidify, again ext. with ether, and wash combined ether exts as directed previously. Slowly distill greater portion of ether, let remainder evap. spontaneously, and proceed as in **27.076**.

27.076 DETERMINATION

Dissolve residue, **27.075**, in small quantity of hot H_2O, and after cooling dil. to definite vol. (usually 50 or 100 ml). If soln is not clear, filter thru dry paper. Dil. aliquots of the soln and treat with 0.5% $FeCl_3$ soln or 2% Fe alum soln until max. color is developed. Generally few drops will suffice.

(The Fe alum soln should be boiled until ppt appears, allowed to settle, and filtered. Acidity of soln is slightly increased in this manner, but soln remains clear for considerable time, and turbidity caused by its diln with H_2O is much less and does not appear so soon as when unboiled soln is used. This turbidity interferes with exact matching of color.)

Compare colors developed with color obtained when std *salicylic acid soln* (contg 1 mg salicylic

acid in 50 ml) is similarly treated, using Nessler tubes or colorimeter. In either case, and especially with $FeCl_3$, avoid excess reagent, although excess of 0.5 ml 2% Fe alum soln may be added to 50 ml of the comparison soln of salicylic acid without negating results.

SULFUROUS ACID

27.077 Qualitative Test (*28*)—**Official**

Add small quantity of S-free Zn and several ml HCl to ca 25 g sample (with addn of H_2O, if necessary) in 200 ml erlenmeyer. H_2S generated in presence of sulfites may be detected with $Pb(OAc)_2$ paper. Traces of metallic sulfides occasionally present in vegetables give same reaction as sulfites under conditions of above test. Verify positive results obtained by this method by Monier-Williams method, **27.078**.

It is always advisable to make quant. detn of sulfites because of possibility of positive test caused by traces of sulfides. Trace should not be considered sufficient indication of presence of SO_2 either as bleaching agent or as preservative.

Total Sulfurous Acid

27.078 *Monier-Williams Method* (*29*)—*Official*

(Applicable in presence of other volatile S compounds)

Connect 750 ml round-bottom Pyrex flask, *B*, (Fig. 59) to sloping reflux condenser, *D*, lower end of which is cut off at angle. (Monier-Williams prefers using upright round-bottom 1.5 L flask with 2 necks.) Pass CO_2 from generator thru dil. Na_2CO_3 soln in *A* to remove Cl. Also connect dropping funnel *K*, to *B* by 3-hole stopper *C*. Use tube *E* to connect upper end of condenser to 200 ml erlenmeyer, *F*, which is followed by Peligot tube, *G*. This delivery tube, *E*, extends to bottom of receiver. One Peligot tube has been found sufficient to catch traces of SO_2 swept thru flask *F*. Use rubber stoppers thruout.

Receiver *F* contains 15 ml pure neutral 3% H_2O_2, and Peligot tube contains 5 ml 3% H_2O_2 soln. H_2O_2 usually contains free H_2SO_4. Start with 30% H_2O_2, dil. somewhat, and neutralize with $Ba(OH)_2$ soln, using bromophenol blue, **4.015(f)**, as indicator. Let settle in cold, filter from $BaSO_4$, det. its concn by $KMnO_4$ titrn, and finally adjust to 3% concn. Bromophenol blue in H_2O_2 remains unaffected for some time.

Connect app., add 300 ml H_2O and 20 ml HCl to flask, and boil short time in current of CO_2. Add food to be tested, adapting procedure to type of food. Add liquids directly thru dropping funnel. In case of easily transferable solids, first cool contents of flask somewhat, taking care to regulate flow of CO_2 to avoid having the H_2O_2 drawn up in delivery tube *E*. Then quickly add the food by removing stopper *C*. (Solid foods such as

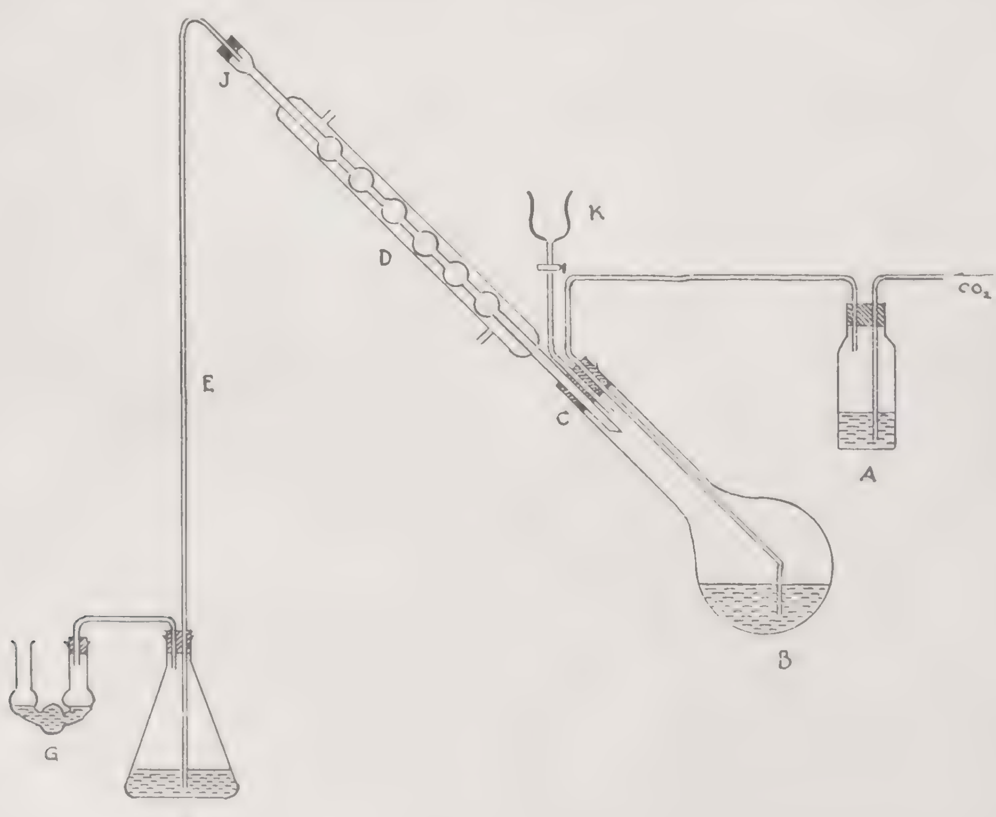

FIG. 59. MONIER-WILLIAMS APPARATUS FOR DETERMINATION OF SULFUROUS ACID

meat and ground dry fruits may be added quickly by first wrapping in filter paper.) With semi-solid foods, requiring more time to add to flask, cool flask contents by gradual immersion in cold H_2O, and quickly wash in food with recently boiled H_2O.

After adding food, boil mixt. 1 hr (1.5 hr for dried fruits) in slow current of CO_2, stopping flow of H_2O in condenser just before end of distn. This causes condenser to become hot and drives over residual traces of SO_2 retained in condenser. When delivery tube just above receiver *F* becomes hot to touch, remove stopper *J* immediately.

Wash delivery tube and Peligot tube contents into flask *F*, and titr. liquid at room temp. with $0.1N$ NaOH, using bromophenol blue as indicator. The NaOH must be stdzd with this indicator. Bromophenol blue is unaffected by CO_2 and also gives distinct color change in cold H_2O_2. 1 ml $0.1N$ NaOH = 3.2 mg SO_2; titrn of small quantities of SO_2 requiring <0.5 ml NaOH is not accurate. Gravimetric detn may be made after titrn, pptn of $BaSO_4$ being carried out at room temp. After letting ppt settle, filter, and wash residual $BaSO_4$ 3 times by decanting with boiling H_2O. Det. blank on reagents, both by titrn and gravimetrically, and correct results accordingly.

27.079 Free Sulfurous Acid (*30*)—**Official**

Treat 50 ml sample in 200 ml flask with ca 5 ml H_2SO_4 (1+3), add ca 0.5 g Na_2CO_3 to expel air, and titr. H_2SO_3 with $0.02N$ I, using few ml starch indicator, **4.004(f)**. Add I soln as rapidly as possible and continue addn until blue color persists several min. 1 ml $0.02N$ I = 0.64 mg SO_2.

THIOUREA (*31*)

Qualitative Tests—Procedure

(Applicable to orange juice)

27.080 *Pentacyanoammonioferroate Test*

(**a**) *Reagent.*—Dissolve 10 g $Na_2Fe(CN)_5NO.2H_2O$ in 40 ml NH_4OH and let stand at ca 0° until all nitrosoferricyanide decomposes (shown when few drops mixt. no longer give red color when added to soln of creatinine in $1N$ NaOH; decomposition is complete after 24 hr). Filter, and add absolute alcohol to filtrate until there is no further pptn of pentacyanoammonioferroate. Collect pentacyanoammonioferroate on filter, wash with absolute alcohol until NH_3-free, dry *in vacuo* over H_2SO_4, and store in desiccator over $CaCl_2$ in dark. Reagent is 1% soln of this salt in H_2O, exposed to light and air 1 day and then stored in brown glass bottle in dark. It gains in potency for several weeks, and can be kept ca 6 months.

(**b**) *Test.*—Ext. orange juice with ca $\frac{3}{4}$ vol. ether, centrifuge, and sep. lower layer. Stir in some Filter-Cel and filter with suction. Keep vac. on short time and agitate soln to remove most of ether. To ca 5 ml filtered soln add 5 drops of the reagent and note color. If blue color does not develop, add I soln, ca $0.1N$, dropwise, shaking after each drop. Blue-green color indicates presence of $CS(NH_2)_2$. (Usually ca 5 drops I soln are necessary to develop max. color; excess I tends to reduce color.)

27.081 *Grote Reagent Test*

To 5–10 ml orange juice, ether-extd and filtered as in **27.080**, add $0.02N$ I dropwise until I color does not immediately disappear. Add few ml of the dil. Grote reagent, **27.083(b)**. Blue-green or blue color developing rather gradually indicates presence of $CS(NH_2)_2$.

Quantitative Methods

Rapid Oxidation Method for Orange Juice—First Action

27.082 APPARATUS

Siphon.—Insert 2 bent glass tubes in 2-hole cork or stopper, one terminating just below stopper (blow tube) and other (siphon tube) long enough to reach bottom of centrifuge bottle when cork with tubes is inserted in mouth of bottle. Attach another glass tube to outside end of siphon tube with flexible rubber tube so that end of outside tube is below end of inside tube. This assembly is used to siphon lower layer from centrifuge bottle, and rate of flow is controlled by squeezing rubber connection. Prep. cap for inner siphon tube by boring hole of same diam. as tube part way thru small cork.

27.083 REAGENTS

(**a**) *Modified Grote reagent.*—Dissolve 0.5 g $Na_2Fe(CN)_5NO.2H_2O$ in 10 ml H_2O in 50 ml erlenmeyer. Weigh 0.5 g $NH_2OH.HCl$ and 1 g $NaHCO_3$. Mix the 2 solids uniformly in small beaker or porcelain dish by gentle grinding with small pestle or flat-end glass rod, crushing any lumps in sample. Brush off rod or pestle and quantitatively transfer mixed solid to the nitroprusside soln with aid of short-stem funnel and brush. Do not agitate flask but let it stand until CO_2 evolution nearly stops. Then swirl to dissolve any remaining $NaHCO_3$. When evolution of CO_2 practically ceases, add 0.10 ml Br (11 small drops). Second evolution of gas occurs. When agitation no longer produces effervescence, dil. to 25 ml with H_2O and filter. Test reagent as follows: Dil. 2 ml as in (**b**); add 1 ml dild reagent to 10 ml soln composed of 5 ml thiourea stock soln (**c**) (dild 10×), 5 ml H_2O, and 1 drop HOAc. Strong blue color should develop in 5 min. If it does not, prep. new reagent and repeat test.

Store at room temp. 5–10 hr to age soln. (Soln should be mahogany brown. If it has greenish cast, it is not as effective and soon loses its value.) This stock soln keeps several weeks when stored in refrigerator.

(b) *Dilute Grote reagent.*—Dil. 1 vol. reagent (a) with 4 vols H_2O. Use 1 ml dild reagent for each detn. The dild reagent keeps 1 day.

(c) *Thiourea stock soln.*—Dissolve 100 mg $CS(NH_2)_2$ in H_2O and dil. to 200 ml.

(d) *Citric acid-potassium citrate soln.*—Dissolve 0.84 g $K_3C_6H_5O_7.H_2O$ and 1 g citric acid in H_2O, and dil. to 100 ml.

(e) *Sulfuric acid.*—$1.00 \pm 0.02N$.

27.084 PREPARATION OF SAMPLE

Juice oranges in ordinary reamer, strain out seeds and pulp, and mix well. Measure 125 ml into 250 ml centrifuge bottle, add 70 ml ether, and shake well 1–2 min. Centrifuge ca 10 min. at 1800 rpm. Cap short end of the siphon (inside bottle), insert into bottle, and lower thru top layer into lower aq. layer. Push off cork cap with glass rod. Lower tube to bottom of bottle, push cork (which carries siphon) into mouth of bottle, and blow in short tube to start flow of liquid. Carefully siphon into beaker as much of lower layer as possible, controlling rate of flow by squeezing on rubber connection. Stop flow when material from center emulsion layer begins to enter tube.

Add teaspoonful of Filter-Cel to siphoned liquid in beaker, stir well, and filter on büchner (7–11 cm) with suction, using Whatman No. 54 or 41-H paper. Measure filtrate with graduate, pour into separator, and add ca $\frac{1}{2}$ its vol. ether. Shake well, let sep., drain lower layer into beaker, add pinch of Filter-Cel, stir well, and filter thru Whatman No. 12 folded filter. Place filtrate in clean, dry suction flask, warm on steam bath to 36°, and apply suction to remove ether.

Pipet 25 ml filtrate (clear or nearly so) into clean 50 ml vol. flask. In 2 similar flasks place 25 ml aliquots of the citric acid-K citrate soln. To one flask add 2 ml of the thiourea stock soln; use other as a blank. To each of the 3 flasks add 5 ml of the $1N$ H_2SO_4. Add $0.1N$ I, **42.016**, slowly, with rotation, to each flask until I color does not disappear; then add 1 ml excess. Let flasks (samples, std, and blank) stand 10 min. at room temp. Now add soln of $NaHSO_3$ (2.5 g/L) to contents of flasks until I color disappears; add 3 or 4 drops excess. Add gradually and slowly to each flask, with swirling, 4 ml 25% NaOAc soln, dil. to vol. with H_2O, and mix. Filter if cloudy. Designate oxidized dild sample as Soln X.

27.085 DETERMINATION

Prep. 2 stds by placing 5 and 10 ml portions of soln from the std flask in test tubes. Dil. first tube to 10 ml by adding 5 ml liquid from blank soln (no thiourea). Place 10 ml portions blank soln and sample Soln X in 2 other test tubes. Pipet 1 ml of the dild Grote reagent into each tube with shaking or stirring. Let tubes stand 1 hr at ca 25°, or 10–15 min. in bath at 45–50°, to develop blue color. Read developed color of solns from each tube (sample, blank, and stds) in neutral wedge photometer or equiv., using filter centered at 610 mμ and 1″ photometer cell. From readings of blank and stds construct curve (linear), plotting photometer readings against ppm of $CS(NH_2)_2$. Oxidized std in flask represents 20 ppm (1 mg/50 ml). 10 ml aliquot therefore represents 20 ppm, and 5 ml 10 ppm.

Slight correction on sample color reading obtained is necessary because of natural color present in Soln X before addn of the Grote reagent. Obtain readings on blank soln and sample Soln X contained in vol. flask, without added reagent, using same photometer cell. Subtract difference between these readings (X − blank) from sample reading with the Grote reagent. Obtain from graph ppm $CS(NH_2)_2$ corresponding to corrected reading. This value $\times 2 = CS(NH_2)_2$ concn in original orange juice.

Method for Frozen Peaches—First Action

27.086 PREPARATION OF SAMPLE

Weigh 200–400 g frozen sample on rough balance (0.1–0.2 g sensitivity) into tared 800 ml beaker. Cut contents of 1 lb package into quarter or eighth portions, select alternate portions for detn, and keep remainder as reserve sample. (Several packages can be composited in this manner if desired.)

Immediately weigh into beaker contg sample, quantity of $NaHSO_3$ soln (2.5 g/L) equal to $\frac{1}{2}$ wt sample (NOTE 1). Stir contents of beaker and pour into high speed blender (or other mixing machine), drain well, and comminute in blender 20–30 sec. Return dild comminuted sample to beaker.

Weigh 150 ± 0.2 g blended sample, transfer to 250 ml vol. flask, and dil. to ca 200 ml with H_2O. Add ca 4 drops *hexyl alcohol* and attach to flask 2-hole stopper (No. 0), carrying small bent glass tube and another straight tube, extending ca 3″ into flask, end of which is drawn to small bore (near capillary). Remove most of air by applying gentle suction to bent tube and shaking flask with rotation. (Bore of small tube should be large enough so that reduction of pressure is not too great.) If froth rises in neck, release vac. for moment. Then continue with suction and rotation until most of air is removed, add 20 ml more of the $NaHSO_3$ soln, and dil. to mark with H_2O. Mix well and pour ca 165 ml into 250 ml centrifuge bottle. Add 50 ml ether to contents of bottle,

rotate few times, stopper, and shake; open once to release pressure; then shake vigorously 1–1.5 min. (If preferred, divide liquid in vol. flask between 2 centrifuge bottles and ext. each with ca 30 ml ether, etc.)

Centrifuge at ca 1800 rpm ca 10 min. Carefully pour off little of top ether layer into beaker; then inclining bottle, push sludge cake toward bottom of bottle with glass rod and pour liquid contents on cotton filter in funnel. To prep. filter, place small cotton pledget in apex of 85–100 mm funnel, and insert piece of absorbent cotton of half thickness (split sheet), ca 3½″ diam.

Pipet 100 ml lower aq. filtrate into 200 ml vol. flask, squeezing cotton on side of funnel with rod if necessary to obtain enough filtrate. Add gradually to contents of flask (from separator), with constant shaking, enough acetone to bring contents to 200 ml mark. As surface of liquid enters neck of flask, stopper, and mix by inverting few times before dilg to mark. Mix, cool to room temp. (in bath if desired), dil. to mark again, and mix well. (Acetone causes some rise in temp.) Let ppt sep. and pour contents of flask into 250 ml centrifuge bottle. Add 1 spoonful of Filter-Cel, stopper, and shake well; then centrifuge ca 8 min. at ca 1800 rpm. Decant off supernatant and filter if turbid. Measure 125 ml clear liquid into 250 ml beaker, add several glass beads, and boil off acetone on steam bath. Then boil down to ca 35 ml on hot plate, remove, and cool to room temp. Add dropwise 15% NaOH soln until alk. and ca 2 drops excess. Add HOAc (1+5) with stirring until just acid and add 2 drops excess.

Transfer liquid quantitatively to 50 ml vol. flask, dil. to mark with H_2O, and mix. Pour contents of flask into small beaker or flask (100–125 ml), add 1 spoonful Filter-Cel, and mix well by stirring or stoppering and shaking. Filter liquid on 12.5 cm folded paper (E. & D. No. 195 is suitable). Pour thru filter again if not clear. Filtrate or final sample soln is designated *F. S.*

27.087 DETERMINATION

Pipet 10 ml of Soln *F. S.*, **27.086**, into 6″ test tube. For quantities of $CS(NH_2)_2$ up to 50 ppm, prep. stds contg 0, 1, 2, and 4 ml portions of 1+9 diln $CS(NH_2)_2$ stock soln, **27.083(c)** (5 mg $CS(NH_2)_2$/100 ml). Add 0.6% Na citrate soln to tubes to make stds to 10 ml; then add 1 drop HOAc (1+5) to each tube (samples and stds). Place stirring rod in each tube and stir up and down to mix, leaving rod in tube. Place tubes in bath or room held at 20–25°.

Add to each tube, with stirring, 1 ml dil. Grote reagent, **27.083(b)** (recently dild). Let tubes stand 60 min. at 20–25° and read blue color of each tube in photometer, using 1″ cell and filter centering at ca 610 mμ. Designate reading of sample as *X*. Construct linear curve from std readings, plotting ppm $CS(NH_2)_2$ (1 ml std soln = 10 ppm $CS(NH_2)_2$ in sample) against photometer readings. Extrapolate std curve up to 50 ppm.

To correct reading *X*, obtained above for natural color present in soln before reagent is added, make readings of Soln *F. S.* with no added reagent in same cell and also that of H_2O. *F. S.* reading − H_2O reading $= d$; $X - d = R$ (corrected reading). From reading *R* obtain ppm $CS(NH_2)_2$ in Soln *F. S.*, using curve. Multiply $CS(NH_2)_2$ thus found by factor 1.065 to correct for vol. increase due to ether and obtain true $CS(NH_2)_2$ content of original sample (NOTE 2). Repeat detn (color development) on smaller aliquot (1–5 ml) for quantities >50 ppm.

NOTE 1: Sample of 200 g is enough to be representative and should be used where portion is to be reserved. Keep unused portion of sample frozen. It is necessary to add the $NaHSO_3$ soln to frozen sample immediately before blending to prevent losses of $CS(NH_2)_2$ due to attack by enzyme systems present. Blender whips air thruout the material, and if enzymes are not inactivated, large losses of $CS(NH_2)_2$ may occur. Enzymes can also be inactivated by plunging frozen sample into boiling H_2O and boiling 3 or 4 min. Action of enzymes is slow in frozen condition if material is unbroken cakes or chunks.

NOTE 2: Correction factor 1.065 is to compensate for solubility of ether in aq. soln. Measurement of 100 ml soln is made after extn with ether. Latter has considerable solubility in aq. solns.

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28. Spices and Other Condiments

SPICES

28.001 Preparation of Sample—Procedure

Grind sample to pass thru sieve with circular openings 1 mm diam. and mix thoroly. Since most spices lack uniformity and have tendency to stratify, use extreme care in weighing out portion for analysis. Stir material thoroly and weigh out 2 g sample, using spoon with ca 2 g capacity. Dip spoonful from center of material, being careful to take ca required quantity so as to avoid adding to or taking from portion on scale pan. In detn of starch in spices by diastase method, further reduce subsample as nearly as possible to impalpable powder.

28.002 Moisture (*1*)—First Action

Clean distg tube receiver and condenser described in **22.004** with $K_2Cr_2O_7$-H_2SO_4 mixt., rinse thoroly with H_2O and then with ca 0.5*N* alc. KOH, and drain 10 min. Before cleaning, remove connecting stopper from condenser, so that it remains dry. Place 40 g spice in distg flask and proceed as in **22.005**.

28.003 Ash (*1*)—Official

(**a**) *Most spices.*—Weigh accurately ca 2 g sample in flat-bottom dish, preferably Pt. Place dish in entrance of open muffle so that sample fumes off without catching fire. Then ignite in muffle 30 min. at 550°, break up ash with several drops H_2O, evap. carefully to dryness, and heat in muffle 30 min. If previous wetting showed ash to be C-free, remove dish to desiccator contg fresh efficient desiccant (H_2SO_4 or anhyd. $Mg(ClO_4)_2$ is satisfactory), let cool to room temp., and weigh soon. If first wetting showed C, repeat wetting and heating until no specks of C are visible; then heat 30 min. after disappearance of C. If C persists, leach ash with hot H_2O, filter thru quant. paper, wash paper thoroly, transfer paper and contents to ashing dish, dry, and ignite in muffle at 550° until ash is white. Cool dish, add filtrate, evap. to dryness on steam bath, and heat in muffle 30 min. Cool, and weigh as previously.

(**b**) *Nutmeg, mace, ginger, and cloves.*—Proceed as in (**a**), but heat at 600°.

(**c**) *Ground mustard or mustard flour.*—Ignite as in (**a**) and heat 30 min. at 550°. Leach ash with hot H_2O, filter, and wash thoroly. Transfer paper and contents to ashing dish, dry, and heat in muffle 30 min. Remove dish, let cool, add 5–10 drops HNO_3, evap. to dryness, and heat in muffle 30 min. Repeat HNO_3 and heating treatment until residue is white. Add filtrate, evap. to dryness, and heat in muffle 30 min. Cool, and weigh as in (**a**).

28.004 Soluble and Insoluble Ash—Official

Proceed as in **29.015**, using ash obtained in **28.003**.

28.005 Ash Insoluble in Acid—Official

Boil H_2O-insol. residue, **28.004**, or total ash, **28.003**, with 25 ml HCl (1+2.5) 5 min., covering dish with watch glass to prevent spattering; collect insol. matter on gooch or ashless filter, wash with hot H_2O until washings are acid-free, ignite until C-free, cool, and weigh.

28.006 Calcium in Ash—Official

Ignite 2–4 g sample as in **28.003**, digest with hot HCl (1+2.5), evap. to dryness, moisten dry residue with dil. HCl, and again evap. to dryness to make SiO_2 insol. Treat residue with 5–10 ml HCl, add ca 50 ml H_2O, let stand on H_2O bath few min., filter, and wash insol. residue with hot H_2O. Det. CaO in combined filtrate and washings as in **6.011**.

28.007 Nitrogen—Official

Proceed as in **2.036**. Use 1 g sample for black or white pepper.

28.008 Nitrogen in Non-Volatile Ether Extract—Official

(For black and white peppers)

Ext. 10 g pepper 20 hr in continuous extn app. with absolute ether, collecting ext. in weighed 250 ml flask. Evap. ether, and dry first at 100° and finally to min. wt at 110°. Det. N in weighed ext. as in **2.036**, digesting in same flask used for extn. Crude piperine = N × 20.36.

28.009 Volatile and Non-Volatile Ether Extract (*2*)—Official

(Not suitable for detn of volatile ether ext. in spices high in volatile oils, such as cloves)

Ext. 2 g ground material 20 hr in continuous extn app. with anhyd. ether. Transfer ethereal

soln to weighed capsule and let evap. at room temp. Store 18 hr over H_2SO_4 and weigh total ether ext. Heat ext. gradually and then to min. wt at 110°. Loss is volatile ether ext.; residue is non-volatile ether ext.

28.010 Alcohol Extract (*3*)—Official

Place 2 g sample in 100 ml vol. flask and fill to mark with alcohol. Stopper, shake at 30 min. intervals during 8 hr, and let stand 16 hr longer without shaking. Filter ext. thru dry paper, evap. 50 ml aliquot filtrate to dryness in flat-bottom dish on steam bath, and heat to min. wt at 110°.

28.011 Copper-Reducing Substances by Direct Acid Hydrolysis—Official

Ext. 4 g sample with five 10 ml portions ether on filter that will retain completely smallest starch granules. Let ether evap. from residue and wash with 150 ml alcohol, 10% by vol.

To avoid clogging of filter by glutinous mass, which may result from washing with H_2O or dil. alcohol, omit all preliminary washings with cassia buds, and cinnamon.

Carefully wash residue from paper into 500 ml flask with 200 ml H_2O, using small wash bottle and gently rubbing paper with tip of finger. Hydrolyze and det. Cu-reducing material as in **22.043**. Express results in terms of starch.

Starch—Official

28.012 *Method I.*

Ext. 4 g finely pulverized sample with ether and 500 ml 10% alcohol as in **28.011**, and det. starch by diastase method, **22.045**.

28.013 *Method II.*
(Applicable to dry mustard)

Treat 2–3 g dry mustard flour as in **28.038**.

28.014 Crude Fiber—Official

Proceed as in **22.040**, and before drying the crude fiber remove all ether extractives by successive washings with ether.

Tannin (*4*)—Official
(For cloves and allspice)

28.015 REAGENTS

(**a**) *Oxalic acid soln.*—0.1*N*. 1 ml = 0.006235 g quercitannic acid or 0.0008 g O absorbed.

(**b**) *Potassium permanganate std soln.*—Dissolve 1.333 g $KMnO_4$ in 1 L H_2O and stdze against (**a**).

(**c**) *Indigo soln.*—Dissolve 6 g Na indigotin disulfonate in 500 ml H_2O by heating; cool, add 50 ml H_2SO_4, dil. to 1 L, and filter.

28.016 DETERMINATION

Ext. 2 g sample 20 hr with anhyd. ether. Boil residue 2 hr with 300 ml H_2O, cool, dil. to 500 ml, and filter. Measure 25 ml of this infusion into 2 L porcelain dish; add 20 ml of the indigo soln and 750 ml H_2O. Add the std $KMnO_4$ soln, 1 ml at time, until blue color changes to green; then add few drops at time until color becomes golden yellow. Similarly titr. mixt. of 20 ml of the indigo soln and 750 ml H_2O. Multiply difference between 2 titrns by desired factor to obtain quercitannic acid or O absorbed.

28.017 Volatile Oil (*5*)—First Action

Prep. sample as in **28.001**, except use No. 20 sieve. Also take precaution to prevent loss of volatile oil due to heating during grinding.

Transfer enough weighed ground material to 1–3 L round-bottom, short-neck flask to yield, if possible, 2–5 ml volatile oil. Add H_2O to fill flask slightly less than half full and mix by swirling. Add glass beads and piece of *carnaüba wax* ca ½" diam. If foaming persists, cool completely and add few drops aq. soln of wetting agent. Connect flask thru appropriate calibrated oil trap, Fig. 60, to long condenser (preferably West type). Clean trap and condenser with cleaning soln just prior to use. Use oil bath as source of heat. Distill until there is no increase in oil content over 1 hr period, but not <4 hr.

With spices contg volatile oils lighter than H_2O and fixed oils heavier than H_2O (*e.g.*, nutmeg), discontinue distn when fraction of oil obtained during 1 hr is heavier than H_2O.

To correct unsatisfactory sepn of oil and H_2O, agitate liquid in trap with Cu wire thru condenser, or tap top of condenser laterally.

Measure oil directly in trap after letting it stand until cool. Report oil as ml/100 g spice. Drain oil layer into g-s. tube or graduate, sepg from H_2O layer. Let oil stand until clear, or dry with min. quantity anhyd. Na_2SO_4, and let settle before detg chemical and physical characteristics. Store in refrigerator.

28.018 Specific Gravity of Volatile Oil—Official

Det. sp. gr. at 25/25° as in **26.003** and **26.004**, using 1 ml Sprengel tube.

28.019 Refractive Index of Volatile Oil—Official—*See* 26.007 and 26.009

28.020 Eugenol in Volatile Oil—First Action

Measure 2 ml volatile oil (transfer pipet) into Babcock milk bottle, **15.030(a)**. Add 20 ml 3% KOH soln, shake mixt. 5 min., heat 10 min. in boiling H_2O bath, remove, and cool to room temp. When liquids sep. completely, add enough KOH soln to bring residual oil within graduated portion of neck and note vol. Calc. % by vol. from difference between vol. sample used and residual oil.

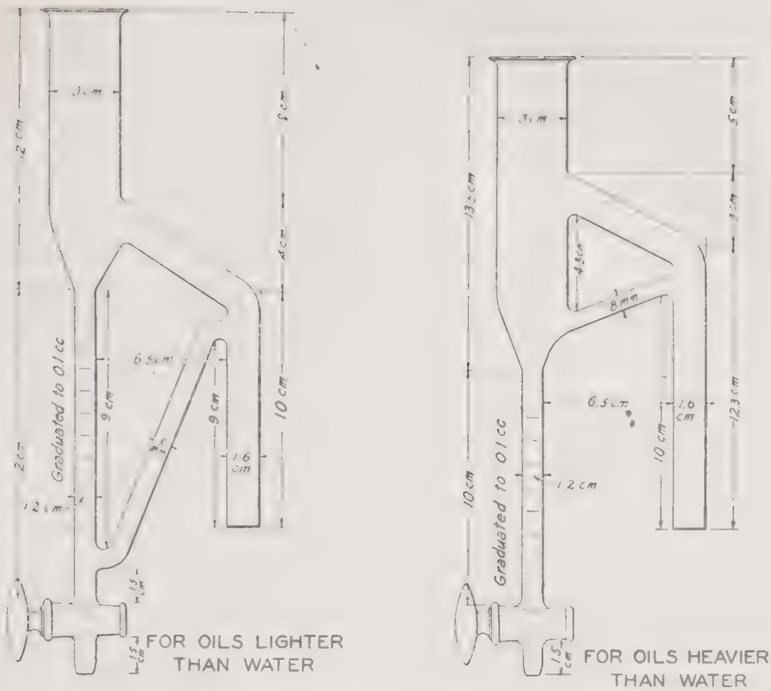

FIG. 60.—TYPES OF OIL SEPARATORY TRAPS

28.021 Volatile Oil and Resin in Ginger (*6*)—First Action

Place 50 g ground ginger in Soxhlet extractor and ext. completely with ether (ca 4 hr). Transfer ext. to 300 ml flask and evap. ether on steam bath until solvent is no longer detected. Add 50 ml H_2O to residue and det. yield of volatile oil (using trap for oils lighter than H_2O), sp. gr., and refractive index, as in **28.017–28.019.**

Transfer residue in flask to separator and ext. resin with ether. Transfer to tared beaker, evap. ether on steam bath, and dry to constant wt in vac. desiccator.

28.022 Volatile Oil in Mustard Seed (*7*)—Official

Place 5 g ground seed (thru No. 20 sieve) in 200 ml flask, add 100 ml H_2O, stopper tightly, and macerate 2 hr at ca 37°. Add 20 ml alcohol and distill ca 60 ml into 100 ml vol. flask contg 10 ml NH_4OH (1+2), taking care that end of condenser dips below surface of soln. Add 20 ml 0.1N $AgNO_3$ to distillate, let stand overnight, heat to boiling on H_2O bath in order to agglomerate Ag_2S, cool, dil. to 100 ml with H_2O, and filter. Acidify 50 ml filtrate with ca 5 ml HNO_3 and titr. with 0.1N NH_4CNS, using 5 ml 10% $FeNH_4(SO_4)_2.12H_2O$ soln as indicator. 1 ml 0.1N $AgNO_3$ = 0.004956 g allyl isothiocyanate.

28.023 Iodine Number of Paprika Oil (*8*)—Official

(Qual. test for presence of foreign oil)

Transfer 10 g well-mixed ground sample to 200 ml g-s. flask and pipet in 100 ml $CHCl_3$, rotating flask while adding first 50 ml. Let stand 1 hr, shake, and filter thru 12.5 cm fluted paper. Pipet off two 10 ml portions $CHCl_3$, using same pipet. Transfer one portion to weighed crystg dish, 50×35 mm, and evap. solvent on steam bath. Dry dish and contents 1 hr at 100°, cool in air, and weigh. Use wt obtained in calcg I number. Transfer other portion to suitable g-s. flask or bottle for detn of I number, **26.017,** allowing 30 min. for halogen absorption. Calc. I number of $CHCl_3$ ext. I number of pure paprika oil thus obtained should be not <130.

Microscopic Examination—Procedure

28.024 GENERAL

Adulterants of vegetable origin in spices are best detected microscopically. General knowledge of vegetable histology and microscopic appearance of spices and spice adulterants is essential. Some std works on these subjects (*9*) are listed in Selected References.

28.025 REAGENTS

(**a**) *Acidified chloral hydrate-glycerol soln.*—Dissolve 270 g chloral hydrate crystals in 150 ml H_2O, 19 ml HCl, and 60 ml glycerol.

(**b**) *Chloral hydrate soln.*—Dissolve 8 parts by wt chloral hydrate crystals in 5 parts H_2O.

(**c**) *Ferric acetate or chloride soln.*—Freshly prepd 1% aq. soln.

(**d**) *Iodine-potassium iodide soln (iodine soln).*—Dissolve 0.5 g I and 1.5 g KI in very small amount of H_2O and dil. to 25 ml.

(**e**) *Iodine-potassium iodide in zinc chloride*

soln.—Dissolve 100 g $ZnCl_2$ in 60 ml H_2O in g-s. bottle and add 20 g KI and 0.5 g I. Leave few I crystals in bottle to insure satn and let soln stand few hr before use. Soln keeps for months. If color developed in tissue is too deep blue, dil. reagent slightly.

(f) *Millon reagent.*—*See* **22.014.**

(g) *Potassium chlorate macerating soln.*—Mix 0.5 g $KClO_3$ with 50 ml HNO_3 (1+1) as needed.

(h) *Potassium hydroxide soln.*—Dissolve 5 g KOH in H_2O and dil. to 100 ml.

(i) *Sudan IV, saturated alcoholic soln.*—Approx. 0.09%.

(j) *Mayer reagent* (*mercuric-potassium iodide soln.*)—Dissolve 1.36 g $HgCl_2$ in 60 ml H_2O and 5 g KI in 10 ml H_2O; mix 2 solns and dil. to 100 ml.

28.026 APPARATUS

(a) *Wide-field stereoscopic microscope.*—Instrument with ca 10 to 60× magnification is useful for preliminary sepn.

(b) *Compound microscope.*—Instrument with ca 100 to 400× magnification. Eyepiece micrometer, mechanical stage, and polarizing microscope are desirable for special types of work.

(c) *Sieves.*—Series of std mesh sieves from No. 10 to 100, and sieve with circular openings 1 mm diam.

(d) *Slides, cover glasses, needles, forceps, etc.*

28.027 PREPARATION OF SAMPLE

Reduce one portion to fine powder in mortar. Sep. another portion into several grades of fineness by sieves of different mesh or by jarring on sheet of paper. In coarser grades, fragments of suspicious nature may often be seen with naked eye or under simple microscope; these should be picked out for subsequent examination under compound microscope.

28.028 EXAMINATION

Mount small quantity ground sample in H_2O and examine under compound microscope with both ordinary and polarized light. This gives general information as to nature of material and serves for detection and identification of starch granules and various tissues. Place small drop of the I-KI soln at edge of cover-glass, draw it into prepn by means of piece of filter paper placed at opposite edge of cover-glass, and examine again. Starch granules are colored blue or blue-black; cellulose, yellow; and proteins, either brown or yellow.

In manner described draw little of the KOH soln under cover-glass and again examine. This treatment gelatinizes starch granules, dissolves proteins, saponifies fats, and in other ways clears prepn. It also imparts reddish color to tannins. If this treatment does not clear tissues satisfactorily, treat fresh portion for short time with acidified chloral hydrate-glycerol soln, heating gently, if necessary, or for some hrs with the chloral hydrate soln.

Examine also crude fiber obtained in chemical analysis, as stone cells and other tissues are shown distinctly in this material.

To isolate stone cells, bast fibers, and other thick-wall cells, macerate portion of sample in $KClO_3$ macerating soln, varying proportions of $KClO_3$ and HNO_3 and heating long enough to secure desired results.

To distinguish cellulose from infiltrated substances (lignin, suberin, etc.), add freshly prepd I-KI in $ZnCl_2$ soln to H_2O mount. Cellulose is colored blue, and infiltrated substances are yellow.

To distinguish fats, oils, essential oils, resins, latex, and wax from other cell contents, add to small amount of tissue on slide 2 drops Sudan IV soln and 2 drops glycerol or acidified chloral hydrate-glycerol soln and heat gently; these substances are stained red. Treat sep. portion tissue with ether, petr. ether, or alcohol. Ether and petr. ether dissolve fats, oils, essential oils, resins, latex, and wax. Alcohol dissolves essential oils and resins but usually affects fats, oils, latex, and wax slowly or not at all.

Test for proteins by warming cautiously on slide with drop of Millon reagent. Proteins are partially decomposed, gradually acquiring brick red color. If it is desired to study form of aleurone (protein) granules, which in some plants are quite as characteristic as starch granules, prep. mount in pure glycerol or oil.

Test for tannins and tissues impregnated with them by adding the $Fe(OAc)_3$ or $FeCl_3$ soln. Both reagents give green or blue color with tannins, but $Fe(OAc)_3$ acts more slowly and is preferred.

Crystals of Ca oxalate (*10*) are recognized by their characteristic forms and by behavior to polarized light. To distinguish Ca oxalate from $CaCO_3$, treat with HOAc, which does not affect oxalate but dissolves carbonate with effervescence. Both are sol. in HCl.

Powd. charcoal and charred shells resist bleaching action of KOH, chloral hydrate, and $KClO_3$ macerating soln.

PREPARED MUSTARD

28.029 **Preparation of Sample—Procedure**

Transfer entire contents of container to dish large enough to permit thoro stirring and make whole mass homogeneous. Preserve in g-s. bottle. Stir well each time before removing portion for analysis.

28.030 **Solids—Official**

Weigh 5 g sample into flat-bottom Pt dish; distribute evenly over bottom of dish with little

H_2O, place on steam bath until mixt. appears dry, and heat in oven at 100° to min. wt.

28.031 Total Chlorides (*11*)—Official

Weigh 3–4 g sample from weighing bottle, place in 300 ml erlenmeyer, and add excess of std 0.1*N* $AgNO_3$ (usually 30 ml is enough). Mix thoroly, add 15 ml HNO_3, and bring to boil on hot plate. Add to boiling mixt. 15 ml 5% $KMnO_4$ soln, 5 ml at time, rotating flask after each addn to mix contents. Add ca 50 ml H_2O and filter into 200 ml vol. flask. Wash filter free of $AgNO_3$ and dil. to mark with H_2O. Mix thoroly and titr. 100 ml aliquot with 0.1*N* KCNS, using 2 ml satd Fe alum soln as indicator. Calc. chlorides as NaCl.

28.032 Ether Extract—Official

Weigh 10 g sample into SiO_2, Al, or porcelain drying dish and mix with ca 30 g sand. Heat on H_2O bath until mixt. appears dry; then finish drying in H_2O oven. Grind until all lumps are broken up, and det. ether ext. by extg 16 hr with anhyd. ether in Soxhlet extractor with Whatman single thickness or other close-texture thimble. Dry ext. 30 min. at 100°, cool, and weigh.

28.033 Total Nitrogen—Official

Det. N as in **2.036**, using 5 g sample.

28.034 Acidity—Official

Weigh 10 g sample into 200 ml vol. flask, dil. to mark with H_2O, shake, filter thru dry paper, and det. acidity in 100 ml by titrn with 0.1*N* alkali, using phthln. Express result as HOAc. 1 ml 0.1*N* alkali = 0.0060 g HOAc.

Sucrose—First Action

28.035 REAGENTS

(**a**) *Ion exchange resins.*—Amberlite IR 120 H, cation ion-exchange resin (Rohm & Haas Co., Philadelphia, Pa.), and Duolite A-4, anion ion-exchange resin (Chemical Process Co., Redwood City, Calif.).

(**b**) *Invertase soln.*—Dil. 8 ml commercial invertase ("Convertit," American Molasses Co., 120 Wall St., New York 5, N. Y., has been found satisfactory for this purpose) and 2 ml HOAc to 250 ml with H_2O. Store in g-s. bottle in refrigerator.

28.036 DETERMINATION

Transfer 10 g sample and 1 g $CaCO_3$ to 250 ml vol. flask with 125 ml of 50% alcohol, mix thoroly, and boil on steam bath 1 hr, using small funnel in neck to condense vapor. Cool, and let stand 2 hr. Dil. to vol. with 95% alcohol, mix thoroly, and transfer to 250 ml centrifuge bottle. Centrifuge 5 min. at 1200 rpm. Pipet 200 ml supernatant into beaker and evap. on steam bath to 30 ml. (Do not evap. to dryness.) A little alcohol in residue does no harm. Transfer to 100 ml vol. flask, and rinse beaker thoroly with H_2O, adding rinsings to flask, and dil. to vol. with H_2O.

Pour entire contents of flask into g-s. erlenmeyer. Add 2 g Amberlite IR 120 H resin and 2 g Duolite A-4 resin. Let stand 2 hr, swirling occasionally. Filter thru folded paper (Whatman No. 12 or equiv.) into g-s. erlenmeyer.

Pipet 50 ml of clarified soln into 100 ml vol. flask. Add 25 ml H_2O and 5 ml invertase soln, and let inversion proceed at 20–25° overnight. Dil. to vol. with H_2O.

Det. reducing sugars, on 25 ml aliquot of clarified soln (before inversion) and 50 ml aliquot of inverted soln, as in **29.038–29.040**.

Calc. sucrose as follows: From table **43.011** obtain sugar after inversion from invert column and sugar before inversion from invert sugar and sucrose column (0.4 g total sugar). Subtract % invert sugar obtained before inversion from that obtained after inversion and multiply difference by 0.95 to obtain % sucrose.

Starch—Official

28.037 REAGENTS

(**a**) *Calcium chloride soln.*—30 g/100 ml soln adjusted to 0.01*N* alky.

(**b**) *Alcoholic sodium hydroxide soln.*—70 ml alcohol + 30 ml 0.1*N* NaOH.

(**c**) *Iodine-potassium iodide soln.*—2 g I + 6 g KI in 100 ml H_2O.

28.038 DETERMINATION

Place 5 g prepd mustard in 500 ml erlenmeyer and pipet in 100 ml of the $CaCl_2$ soln, swirling flask gently until all lumps are broken. Add calcd quantity 1*N* NaOH to neutralize acid in wt prepd mustard taken for analysis. Add glass beads. Connect to reflux condenser, first wetting inside of condenser and stopper with H_2O and draining 1 min. Heat gently (on asbestos board with center hole) to avoid initial foaming, and boil 15 min.

Leaving condenser connected, cool flask to room temp. in pan of cold H_2O. Remove flask, stopper, and shake vigorously. Pour contents into centrifuge bottle and whirl at 1500 rpm 5 min. Withdraw as much as possible of partially clarified middle layer (ca 75 ml) and filter thru 11 cm circle of absorbent cotton ca 5 cm thick placed in 60° funnel. Pipet 50 ml filtrate into second centrifuge bottle contg 150 ml alcohol, stopper, and shake vigorously several min. Centrifuge at 1500 rpm until clear (ca 5 min.).

Decant liquid thru asbestos pad in Caldwell crucible, using suction, without transferring starch to crucible. Transfer pad to same centrifuge bottle, and rinse all particles adhering to crucible into bottle with H_2O. Add several glass

beads and H_2O to ca 100 ml. Stopper and shake bottle vigorously until ppt is as finely dispersed as possible. Add slight excess of the I-KI soln (2–3 ml) and 30 ml satd $(NH_4)_2SO_4$ soln. Stopper and shake bottle. Rinse particles adhering to stopper into bottle, and centrifuge until clear.

Decant supernatant, with suction, thru asbestos pad in Caldwell crucible. Add 50 ml of the alc. NaOH soln to ppt in centrifuge bottle. Stopper and shake vigorously. Wash stopper with 70% alcohol. Centrifuge and decant supernatant thru same pad as before. Repeat treatment with the NaOH soln until practically all blue color disappears (usually 2–3 treatments). Without centrifuging, transfer contents of bottle to Caldwell crucible, using 70% alcohol. Aspirate until pad is dry; then transfer pad to 500 ml Kjeldahl flask. Rinse bottle and crucible with 10 ml HCl (sp. gr. 1.1029) followed by five 10 ml portions H_2O, carefully removing all adhering particles. Attach Kjeldahl flask to reflux consdenser, first adding glass beads to lessen bumping. Place on asbestos board with center hole and boil 1 hr. Cool, neutralize with NaOH (1+1) (Me orange), and filter into 200 ml vol. flask; rinse flask and filter thoroly, and dil. to vol. with H_2O. Mix well, and det. dextrose in 50 ml aliquot by **29.039.** (Blank on Fehling soln should be not >0.3 mg.)

$$\% \text{ starch} = [\text{g dextrose} \times 0.9(100 + A + B) \times 8]/\text{wt sample},$$

where A = ml $1N$ NaOH used to neutralize acidity, **28.034,** and B = g H_2O in sample taken (calcd from solids, **28.030**).

28.039 Crude Fiber (*12*)—Official

Weigh 10 g sample and transfer to 8 oz nursing bottle with 50 ml alcohol, stopper, and shake vigorously. Add 40 ml ether, shake, and let stand ca 5 min., shaking occasionally. Centrifuge and decant alcohol-ether mixt. Treat twice more with 40 ml portions ether, shaking, centrifuging, and decanting as before. Rest bottle on its side for short time, without heat, to let most of ether evap. Transfer material to 500 ml erlenmeyer, using 200 ml boiling H_2SO_4, **22.038(a),** and proceed as in **22.040,** but in addn wash fiber with successive portions of ether before drying and weighing.

If preferred, treat sample with alcohol and ether in small beaker, transfer to hardened 11 cm filter paper, wash several times with ether, and transfer to 500 ml erlenmeyer with 200 ml boiling H_2SO_4.

28.040 Preservatives—Official—*See* Chap. 27

DRESSINGS FOR FOODS (*13*)

28.041 Preparation of Sample—Procedure

(**a**) *Semi-solid and emulsified dressings.*—Before removing any portion of sample for analysis, transfer to suitable container, such as glass fruit jar, of larger capacity than vol. of sample, and mix with spatula until homogeneous (2–3 min. should be enough). Repeat mixing before each subsequent portion is removed for analysis if sample has stood for any appreciable time. For the various detns, take ca quantity directed and weigh. (Light 100 ml flask fitted with straight glass tube and oversized rubber bulb makes suitable weighing bottle.)

(**b**) *Separable dressings, small containers.*—Weigh bottle contg sample. Shake bottle 1 min., empty contents into high speed blender, and let bottle drain 1 min. Weigh empty bottle to det. wt sample. Add 0.20 g egg albumen powder/100 g sample, cover blender, and stir 5 min.; then transfer to suitable container of capacity larger than sample vol. Shake sample ca 20 times and stir with spatula or spoon ca 20 times before each portion is removed for analysis. Make all weighings immediately after sample prepn. Correct results for added emulsifier.

(**c**) *Separable dressings, large containers.*—Stir contents thoroly, adding 0.20 g egg albumen powder/100 g sample. Mechanical stirrer of double-beater type is satisfactory. Continue stirring until powder is well dispersed thruout sample. Add sample in portions to high speed blender and stir each portion ca 5 min. Transfer emulsified portions to jar of ca same size as original container and stir entire contents of sample to insure uniform mixt. Transfer portion of prepd sample to suitable jar (ca 1 pint). Proceed as in (**b**), beginning "Shake sample ca 20 times . . ."

28.042 Total Solids—Official

Use 2 g sample and proceed as in **16.003.**

28.043 Reducing Sugars Before Inversion—Official

Weigh 20 g sample into wide-mouth 4 oz bottle and ext. oil by adding ca 80 ml petr. ether, shaking, and centrifuging. Draw off as much as possible of petr. ether soln (conveniently done by using suction and short-stem pipet), and repeat treatment with petr. ether until all oil is removed (indicated by absence of color in solvent; usually 4 extns are required). Reserve ether soln for identification of oil. Remove petr. ether from residue with current of air and transfer residue with H_2O to 100 ml vol. flask. Add 5–10 ml *fresh soln of HPO_3* (remove any white coating on HPO_3 by rinsing with H_2O; dissolve 5 g transparent lumps

or sticks in cold H_2O, and dil. to 100 ml), mix thoroly, dil. to vol., and filter. Transfer 80 ml filtrate, or as large aliquot as possible, to 100 ml vol. flask; neutralize with NaOH soln (1+1), using phthln; cool, dil. to mark, and det. reducing sugars on aliquot as in **29.039.** Calc. to invert sugar.

With dressings, particularly those contg starch, that cannot be clarified by above method, remove oil as in **15.029,** using 1 ml NH_4OH and 5 ml alcohol/g sample; transfer residue to 250 ml vol. flask with alcohol, 50% by vol., and proceed as in **22.041** and **29.039.**

28.044 Reducing Sugars After Inversion—Official

Invert aliquot of soln, **28.043,** as in **29.026**(b) or **(c)**, nearly neutralize with NaOH soln (1+1), and det. reducing sugars in inverted soln as in **29.039.** Calc. to invert sugar from **43.011** or **43.012.**

28.045 Sucrose—Official

Subtract % invert sugar obtained before inversion, **28.043,** from that obtained after inversion, **28.044,** and multiply difference by 0.95.

28.046 Total Acidity—Official

Weigh ca 15 g sample into 500 ml erlenmeyer, dil. to ca 200 ml, and shake until all lumps are thoroly broken up. Titr. with 0.1*N* NaOH, using phthln, and calc. as HOAc. In order to recognize end point, have duplicate sample at hand so that, by comparison, first change of color may be noted.

28.047 Total Nitrogen—Official

Weigh ca 15 g sample into 500 ml Kjeldahl flask and place on steam bath until egg is thoroly cooked and oil seps readily. Cool, and add ca 50 ml petr. ether; mix, and pour off petr. ether thru small filter. Repeat petr. ether treatment twice, rinsing out as much oil as possible. Wash filter with petr. ether and add filter paper to sample in flask. Det. N, using 50 ml H_2SO_4 (more, if necessary) for digestion, as in **2.036.**

28.048 Total Phosphorus—Official

Use 10 g sample and proceed as in **16.019**(a) and **16.020,** except use Pt dish in place of beaker and burn off oil before ashing in muffle.

28.049 Total Fat—Official

Mix sample thoroly and weigh ca 1 g accurately, by difference, into Mojonnier tube. Add 10 ml HCl, shake, set tube in H_2O bath heated to 70°, and bring to boiling. Boil 30 min., shaking tube thoroly every 5 min. Remove from H_2O bath, add H_2O to fill lower bulb of tube (but not neck), and cool to room temp.

To mixt. in Mojonnier tube add 25 ml ether and shake vigorously at least 1 min. Add 25 ml petr. ether and again shake vigorously at least 1 min. To break emulsion centrifuge 5–10 min. at ca 300 rpm. Pour off ether-fat soln into flask, contg porcelain chips or glass beads, that has been dried at 100°, allowed to cool in air to constant wt, and weighed against similar flask similarly treated as counterpoise. Rinse off mouth of tube with small quantity ether after each decantation, letting ether run into flask. Repeat ether extns twice, using only 15 ml of each ether for second and third extns. Again shake vigorously after addn of each ether and centrifuge. If necessary in order to pour off all ether-fat soln after first extn, add more H_2O prior to second decantation.

Slowly evap. combined ether solns in flask, dry ca 90 min. at 100° (placing counterpoise in oven at same time), cool in air to constant wt, and weigh.

28.050 Identification of Oil—Official

Proceed as in Chap. **26,** using oil obtained by evapg petr. ether exts from detn of reducing sugars, **28.043.**

28.051 Gums in Mayonnaise and French Dressing (*14*)—Official

(Not applicable in presence of starch)

Transfer 100 g sample to 250 ml beaker, add 35–40 ml hot H_2O, and mix thoroly. Heat to 65–70° in H_2O bath, add 10 ml *50% trichloroacetic acid soln,* and maintain at 65–70° until emulsion shows signs of breaking (in no case >10 min.). Transfer mixt. to 8 oz nursing bottle and insert pipet guard (*15*) (wide-bore glass tube long enough to reach almost to bottom of centrifuge bottle, with lower end loosely stoppered; tube is held in place by slotted rubber stopper). Centrifuge 15–20 min. at ca 1200 rpm. (This should sep. mixt. into lower aq. layer and upper oily layer, with layer of curd between. If sepn does not occur, add 30–40 ml toluene, mix, and repeat centrifuging.) Using pipet inserted thru pipet guard, remove as much of aq. layer as possible and filter it into 600 ml beaker. Add 5 vols alcohol and let mixt. stand overnight to ppt gums.

Decant or pipet off enough alcohol to leave not >225 ml, transfer contents of beaker to 8 oz nursing bottle, centrifuge until gum settles to bottom, and decant supernatant alcohol as completely as possible. Dissolve residue in not >50 ml hot H_2O, add 1 or 2 ml HOAc, and ppt by adding alcohol to 8 oz mark on nursing bottle. Let stand overnight, or until ppt flocculates, centrifuge at 1200 rpm, and decant alcohol. (Heavy flocculent ppt at this point indicates presence of significant quantity of gum. Slight

ppt should not be considered positive test for gums, as spices present in most mayonnaises and french dressings usually give such a ppt.) Confirm presence of gums as follows:

Add 35 ml hot H_2O to ppt in nursing bottle, transfer to small beaker, add 5 ml HCl, and boil gently 2 min. to hydrolyze gums to sugars. This soln may now be used for various qual. tests for monosaccharide sugars, as follows:

(**a**) *Copper reduction test.*—Transfer 1 ml hydrolyzed gum soln to test tube, neutralize to litmus paper with ca 2*N* NaOH, and remove paper. Add 5 ml Benedict qual. soln, **15.155**(**a**), and boil vigorously 1–2 min. Let cool spontaneously. Voluminous ppt, which may be green, yellow, or red, indicates reducing sugars.

(**b**) *Molisch test.*—Transfer 5 ml hydrolyzed gum soln to test tube, and add 2 drops *15% alc. α-naphthol soln.* Incline tube and slowly pour down inner side 3–5 ml H_2SO_4 so that 2 layers do not mix. Reddish-violet zone at point of contact indicates carbohydrates. (5% alc. thymol soln may be substituted for α-naphthol.)

Gums in Salad Dressing (*16*)—First Action

(Applicable in presence of starch)

28.052 REAGENTS

(**a**) *Calcium chloride soln.*—Sp. gr. 1.2 at 20°. If cloudy, let soln stand so insol. matter may ppt, and then filter.

(**b**) *Iodine soln.*—*See* **28.037**(**c**).

28.053 SEPARATION OF GUMS FROM STARCH

Defat 50 g salad dressing by heating on steam bath in 250 ml beaker until fat seps, cool, and ext. with petr. ether until last ether ext. is colorless. Make alk. with $MgCO_3$ (2–2.5 g), testing with pH test paper. Heat mixt. in H_2O bath at 80° until residual ether and CO_2 are expelled. Then add 100 ml of the $CaCl_2$ soln and heat in boiling H_2O bath bath 30 min., stirring occasionally. Pour into 250 ml Pyrex centrifuge bottle, centrifuge, and decant as much of supernatant as possible into 250 ml separator. Add 10 ml of the $CaCl_2$ soln to residue in bottle and shake well. Centrifuge and decant supernatant as before into separator. Swirl funnel gently and let oil sep. Drain all material below oil into another 250 ml Pyrex centrifuge bottle. Centrifuge and filter supernatant thru 11 cm büchner fitted with Whatman No. 1 or equiv. paper precoated with layer of Celite filter-aid or equiv. Collect filtrate in beaker within bell jar. Add 10 ml of the $CaCl_2$ soln to residue in centrifuge bottle, shake well, centrifuge, and decant supernatant onto filter in büchner. Wash filter with enough $CaCl_2$ soln so that total vol. filtrate is ca 110 ml.

Add slowly, with stirring, 20 ml I soln to the clear ext. to ppt starch-iodide. I should be present in considerable excess over amount required to react quantitatively with the starch. Considerable amounts of reducing substances are present, which must be satisfied before starch can be quantitatively sepd. Add small quantity of Celite filter-aid and let starch-iodide, which seps in finely divided condition, stand ca 1 hr. Filter by suction thru 11 cm Whatman No. 1 or equiv. paper, precoated with adequate layer of Celite. Use wire screen under paper to aid filtration. Do not wash pad. Test for excess I in filtrate with starch-iodide paper or starch soln. This test must be positive to insure removal of all starch. To brown filtrate add 4 vols 95% alcohol and let stand overnight.

Centrifuge off pptd crude gum. Wash twice with 70% alcohol. If possible, gum should be transferred into centrifuge bottle, but in some cases gum adheres so firmly to wall of beaker that it can only be rinsed until washings are clear.

Heat on steam bath or in oven at 100° until alcohol is removed. Dissolve residue in 20 ml H_2O by heating in H_2O bath until no more material dissolves. Use rubber policeman to assist soln. (Be sure gum is dissolved or it will be lost here.) Centrifuge to remove any insol. material. Decant supernatant into another 250 ml centrifuge bottle; add 1 drop HOAc and 1 drop $CaCl_2$ soln; and reppt with 4 vols 95% alcohol. Let stand at least 1 hr or overnight. Centrifuge and wash ppt twice with 70% alcohol by shaking well and centrifuging.

Again drive off alcohol with aid of gentle stream of air by heating in hot H_2O bath and dissolve ppt in 10 ml hot H_2O, using rubber policeman. (Heed warning in preceding par.) Centrifuge to remove any insol. material and decant into 50 ml heavy duty Pyrex centrifuge tube. (Short cone type is less liable to break.) Adjust vol. to 10 ml; add 1 drop HOAc and 1 drop $CaCl_2$ soln; and reppt with 40 ml alcohol. Let stand 1 hr, centrifuge, and wash with 70% alcohol as before. Heavy flocculent ppt at this point indicates presence of gums. Very small quantity of ppt adhering to walls of centrifuge tube or appearing as mere turbidity is to be disregarded, as spice gums present in most salad dressing usually give such ppt.

28.054 DETECTION OF GUM

To confirm presence of gums, remove residual alcohol by gentle heating in hot H_2O bath, dissolve residue in 10 ml hot H_2O, and centrifuge to remove any insol. material. Decant supernatant into 10 ml graduated cylinder, dil. to 10 ml with H_2O, and mix. To 1 ml of this soln add 1 or 2 drops basic $Pb(OAc)_2$ reagent, **29.021**(**a**), 1 drop at time. Immediate flocculent, curdy, or gelatin-

ous ppt is confirmation of presence of gums. Ppt may form on standing but this is to be disregarded.

Starch—Official

28.055 REAGENTS—*See* **28.037**

28.056 DETERMINATION

Det. total acidity of prepd sample as in **28.046.** Place 4–5 g prepd sample in 500 ml erlenmeyer and add calcd quantity 0.1*N* NaOH, *A*, to neutralize acid in wt sample taken. Pipet in 100 ml of the $CaCl_2$ soln, stopper flask, and swirl gently until all large lumps of dressing are broken up. Continue as in **28.038,** line 5, beginning "Add glass beads." Calc. % starch from formula:

$$\% \text{ starch} = [\text{g dextrose} \times 0.9(100 + A + B) \times 8]/\text{wt sample},$$

where B = g H_2O in sample taken (calcd from solids, **28.042**).

VINEGARS (*17*)

(Unless otherwise directed, express results as g/100 ml)

28.057 Organoleptic Examination—Procedure

Note appearance, color, odor, and taste. Neutralize portion of sample with NaOH soln and note odor and taste. Ext. neutralized vinegar with ether, evap. ether ext., and note odor and taste of residue. (Spices and pungent materials are indicated by characteristic odors and tastes.) Evap. portion of sample on H_2O bath. Odor of material as last of volatile matter evaps and appearance and taste of residue give information as to source and character of vinegar.

28.058 Preparation of Sample—Procedure

Mix thoroly and filter thru rapid paper.

28.059 Solids—Official

Measure 10 ml sample into weighed 50 mm diam., flat-bottom Pt dish, evap. on boiling H_2O bath 30 min., and dry exactly 2.5 hr in H_2O oven at temp. of boiling H_2O. Cool in desiccator and weigh. (To obtain concordant results it is necessary to use dish of size and shape stated and to dry exactly time specified.)

28.060 Ash—Official

Measure 25 ml sample into weighed Pt dish, evap. to dryness on H_2O or steam bath, and heat in muffle 30 min. at 500–550°. Break up charred mass in Pt dish, add hot H_2O, filter thru ashless paper, and wash *thoroly* with H_2O. Return paper and contents to dish, dry, and heat 30 min. at ca 525°, or until all C is burned off. Add filtrate, evap. to dryness, and heat 15 min. at ca 525°. Cool in desiccator and weigh (wt *A*). Reheat in muffle 5 min. at ca 525°, and cool not >1 hr in desiccator contg efficient desiccant. Put no more than 2 dishes, preferably only 1, in desiccator at one time. Place wt *A* on balance pan before removing dish from desiccator, and weigh rapidly to mg. Calc. total ash from last wt.

28.061 Soluble and Insoluble Ash—Official

Treat ash, **28.060,** as in **29.015.**

28.062 Alkalinity of Soluble Ash—Official

Proceed as in **29.016,** using sol. ash obtained in **28.061.** Express result as number ml 1*N* acid required to neutralize sol. ash from 100 ml vinegar. If relationship of ash to alky of sol. ash is abnormal, study composition of ash, especially as to content of chlorides, sulfates, phosphates, and alkalies (*18*).

28.063 Soluble Phosphorus (*19*)—First Action

Proceed as in **2.019** or **2.022,** or **20.033,** using soln obtained in **28.062.** If either volumetric or colorimetric method is used, stdze with sample of known phosphate content. Express results as mg P_2O_5/100 ml vinegar.

28.064 Insoluble Phosphorus (*19*)—First Action

Dissolve H_2O-insol. ash, **28.061,** in ca 50 ml boiling HNO_3 (1+8) (use 25 ml H_2SO_4 (1+9) for colorimetric method) and proceed as in **2.019** or **2.022,** or **20.033.** If either volumetric or colorimetric method is used, stdze with sample of known phosphate content. Express result as mg P_2O_5/100 ml vinegar.

28.065 Total Phosphorus (*19*)—Official

Dissolve ash, **28.060,** or both sol. and insol. ash, **28.061,** in ca 50 ml boiling HNO_3 (1+8) (use 25 ml H_2SO_4 (1+9) for colorimetric method) and proceed as in **2.019** or **2.022,** or **20.033.** If either volumetric or colorimetric method is used, stdze with sample of known phosphate content. Express result as mg P_2O_5/100 ml vinegar. If desired, digest vinegar as in **20.032,** instead of using ash from **28.060.**

28.066 Total Acids—Official

Dil. 10 ml sample with recently boiled and cooled H_2O until it appears slightly colored and titr. with 0.5*N* alkali, using phthln. 1 ml 0.5*N* alkali = 0.0300 g HOAc.

28.067 Non-Volatile Acids—Official

Measure 10 ml vinegar into 200 ml porcelain casserole, evap. just to dryness, add 5–10 ml H_2O, and again evap.; repeat until at least 5 evapns

have been made. Add ca 200 ml recently boiled and cooled H_2O, and titr. with $0.1N$ alkali, using phthln. 1 ml $0.1N$ alkali = 0.00600 g HOAc.

28.068 Volatile Acids—Official

Subtract quantity non-volatile acids, **28.067,** from quantity total acids, **28.066.**

28.069 Total Reducing Substances Before Inversion—Official

Measure 25 ml sample into 50 ml vol. flask and add enough NaOH soln (1+1) to nearly neutralize acid. Cool, dil. to mark with H_2O, and det. reducing substances in 20 ml of the soln as in **29.039.** If quantity of reducing substances is very small, use 40 ml. Calc. result as invert sugar (for malt vinegar as dextrose).

28.070 Total Reducing Substances After Inversion—Official

Invert 25 ml sample in 50 ml vol. flask with 5 ml HCl, as in **29.026(b)** or **(c)**. Nearly neutralize with NaOH soln (1+1) and det. reducing substances as in **29.039.**

28.071 Non-Volatile Reducing Substances (Sugar)—Official

(Useful in calcg non-sugar solids)

Evap. 50 ml sample on steam or H_2O bath to sirupy consistency, add 10 ml H_2O, and evap. again. Repeat with 10 ml H_2O. Transfer residue to 100 ml vol. flask with ca 50 ml warm H_2O. Cool; invert with 10 ml HCl as in **29.026(b)** or **(c)**; nearly neutralize with NaOH soln (1+1); cool, dil. to mark with H_2O, and det. reducing substances in 20 ml or 40 ml, depending on quantity present, as in **29.039.** Calc. result as invert sugar (for malt vinegar as dextrose). If results for total reducing substances before and after inversion show absence of sucrose, inversion may be omitted.

28.072 Volatile Reducing Substances (*20*)—Procedure

When sucrose is absent, subtract quantity of non-volatile reducing substances, **28.071,** from mean of total reducing substances before inversion, **28.069,** and after inversion, **28.070.** When sucrose is present, subtract quantity of non-volatile reducing substances, **28.071,** from quantity of total reducing substances after inversion, **28.070.**

28.073 Alcohol—Official

Measure 100 ml sample into round-bottom distn flask. Make faintly alk. with NaOH soln (1+1), distill almost 50 ml, dil. to 50 ml at temp. of sample, and det. sp. gr. at 20/20° with pycnometer, **9.011.** Obtain % by vol. from **43.021.** Undue foaming may be obviated by adding small piece of paraffin, free from volatile constituents.

Glycerol (*21*)—Official

28.074 REAGENTS

(**a**) *Strong potassium dichromate soln.*—Dissolve 74.55 g dry, recrystd $K_2Cr_2O_7$ in H_2O; add 150 ml H_2SO_4; cool, and dil. with H_2O to 1 L at 20°. 1 ml of this soln = 0.01 g glycerol. Because of high coefficient of expansion of this strong soln it is necessary to make all volumetric measurements of soln at same temp. as that at which it was dild to vol.

(**b**) *Dilute potassium dichromate soln.*—Measure 25 ml (**a**) at 20° into 500 ml vol. flask and dil. to mark with H_2O at room temp. 20 ml of this soln = 0.01 g glycerol.

(**c**) *Ferrous ammonium sulfate soln.*—Dissolve 30 g $FeSO_4.(NH_4)_2SO_4.6H_2O$ in H_2O, add 50 ml H_2SO_4, cool, and dil. with H_2O to 1 L at room temp. 1 ml of this soln = ca 1 ml (**b**). As its value changes slightly from day to day, stdze against (**b**) whenever used.

(**d**) *Diphenylamine indicator.*—Dissolve 1 g diphenylamine in 100 ml H_2SO_4.

(**e**) *Retarder.*—Dil. 150 ml H_3PO_4 with 600 ml H_2O, and add 250 ml H_2SO_4.

(**f**) *Milk of lime.*—Place 150 g CaO, selected from clean hard lumps, prepd preferably from marble, in large porcelain or iron dish; slake with H_2O, cool, and add enough H_2O to make 1 L.

(**g**) *Silver carbonate.*—Dissolve 0.1 g Ag_2SO_4 in ca 50 ml H_2O, add excess of Na_2CO_3 soln, let ppt settle, and wash with H_2O several times by decantation until washings are practically neutral. Prep. this reagent immediately before use.

28.075 DETERMINATION

Make evapns on H_2O bath held at 85–90°. Area of dish exposed to bath should not be greater in circumference than that covered by liquid inside.

Evap. 100 ml vinegar to 5 ml, add 20 ml H_2O, and again evap. to 5 ml to expel HOAc. Treat residue with ca 5 g 40-mesh sand and 15 ml of the milk of lime, and evap. almost to dryness with frequent stirring, avoiding formation of dry crust or evapn to complete dryness. Treat moist residue with 5 ml H_2O; rub to homogeneous paste; add slowly 45 ml absolute alcohol, washing down sides of dish to remove adhering paste; and stir thoroly. Heat mixt. on H_2O bath, with constant stirring, to incipient boiling; transfer to suitable vessel and centrifuge.

Decant clear liquid into porcelain dish and wash residue with several small portions hot alcohol, 90% by vol., by centrifuging. (If centrifuge is not available, decant liquid thru folded paper into porcelain dish. Wash residue re-

peatedly with small portions hot 90% alcohol, twice by decantation, and then by transferring all material to filter. Continue washing until filtrate equals 150 ml.) Evap. to sirupy consistency, add 10 ml absolute alcohol to dissolve residue, and transfer to 50 ml g-s. cylinder, washing dish with successive small portions absolute alcohol until vol. of soln is 20 ml. Add three 10 ml portions anhyd. ether, shaking thoroly after each addn. Let stand until clear, pour off thru filter, and wash cylinder and filter with mixt. of 2 vols absolute alcohol and 3 of anhyd. ether. If heavy ppt forms in cylinder, centrifuge at low speed, decant clear liquid, and wash with three 20 ml portions of the alcohol-ether mixt., shaking mixt. thoroly each time and sepg ppt by centrifuging. Wash paper with the alcohol-ether mixt. and evap. filtrate and washings on H_2O bath to ca 5 ml; add 20 ml H_2O, and again evap. to 5 ml; again add 20 ml H_2O and evap. to 5 ml; finally add 10 ml H_2O and evap. to 5 ml. These evapns are necessary to remove all ether and alcohol, and when conducted at 85–90° they result in no loss of glycerol if concn of latter is $<50\%$.

Transfer residue with hot H_2O to 50 ml vol. flask, cool, add Ag_2CO_3 prepd from 0.1 g Ag_2SO_4, shake, and let stand 10 min. Add 0.5 ml basic $Pb(OAc)_2$ soln, **29.021(a)**; shake occasionally, and let stand 10 min. Dil. to mark, shake well, and filter, rejecting first portion of filtrate. Pipet 25 ml clear filtrate into 250 ml vol. flask.

Add 1 ml H_2SO_4 to ppt excess Pb and then 30 ml of the strong $K_2Cr_2O_7$ soln. Add carefully 24 ml H_2SO_4, rotating flask gently to mix contents and avoid violent ebullition, and then place in *boiling* H_2O bath exactly 20 min. Remove flask from bath, dil., cool, and dil. to mark at room temp., using enough strong $K_2Cr_2O_7$ soln to leave excess of ca 12.5 ml at end of oxidation. (Quantity given above, 30 ml, is enough for ordinary vinegar contg 0.35 g or less glycerol/100 ml.)

Stdze the $Fe(NH_4)_2(SO_4)_2$ soln by pipetting 20 ml into 250 ml beaker and adding 20 ml of the retarder, 4 drops of the indicator, and ca 100 ml H_2O. Titr. with the dil. $K_2Cr_2O_7$ soln until liquid assumes dark green color; then add slowly dropwise, stirring continuously, until color changes from blue-gray to deep violet. Designate ml dil. $K_2Cr_2O_7$ soln used as a. In place of the $K_2Cr_2O_7$ soln, substitute buret contg the oxidized glycerol and excess strong $K_2Cr_2O_7$ soln, and titr. 20 ml of the $Fe(NH_4)_2(SO_4)_2$ soln as before, designating ml used as b. From figures obtained calc. glycerol by following formula:

$$G = [D - (250a/20b)]0.02,$$

where G = g glycerol/100 ml vinegar, and D = ml strong $K_2Cr_2O_7$ soln used to oxidize glycerol.

28.076 Color—Official

Det. depth of color in Lovibond tintometer by good reflected daylight, using ½ or 1″ cell and the brewer's scale. Report result in terms of ½″ cell and so state.

28.077 Polarization (*22*)—First Action

Whenever possible, polarize in 200 mm tube without decolorizing. Report result on basis of 200 mm tube in °S, **29.020(a)**. When necessary, decolorize as follows:

(**a**) To 50 ml sample add measured quantity of satd neutral $Pb(OAc)_2$ soln, avoiding excess of Pb; filter, remove Pb with powd. anhyd. K oxalate, and filter. Polarize and correct for diln with $Pb(OAc)_2$ soln.

(**b**) To 50 ml sample add decolorizing C, avoiding excessive quantity or length of treatment. Filter thru double paper and polarize.

28.078 Sulfates—Official

To 100 ml sample add 2 ml ca 1N HCl, heat to boiling, and add 10 ml hot $BaCl_2.2H_2O$ soln (1 g/100 ml), dropwise. Continue boiling 5 min., keeping vol. ca constant by adding hot H_2O as required. Let mixt. stand until supernatant is clear (overnight is convenient, but this time should not be exceeded). Filter on ashless paper or weighed Munroe crucible (*23*). Wash Cl-free with hot H_2O, dry, ignite at low red heat, cool, and weigh. Express result as mg SO_3/100 ml vinegar.

28.079 Dextrin (Qualitative Test)—Procedure

Evap. 100 ml sample to ca 15 ml. Add slowly, and with constant stirring, 200 ml alcohol and let stand overnight. Sep. ppt, preferably by centrifuging, and wash with 80% alcohol. Dissolve in min. quantity H_2O and det. optical rotation. Distinct optical rotation indicates dextrin. Treat soln with several drops I soln of ca same color intensity. Formation of reddish-brown color indicates dextrin.

28.080 Preservatives—Official—*See* Chap. 27

Permanganate Oxidation Number (*24*)—Official

(For differentiating between vinegar and commercial HOAc)

28.081 REAGENTS

(**a**) *Potassium permanganate soln.*—31 g/L. Prep. according to **42.023**; stdzn is unnecessary.

(**b**) *Sodium thiosulfate.*—0.5N. Accurately stdze against $K_2Cr_2O_7$ as in **42.036**, except use ca 0.5 g $K_2Cr_2O_7$, 10 g KI, 10 ml HCl, and 90 ml H_2O.

(**c**) *Potassium iodide soln.*—Dissolve 50 g KI

in 100 ml H_2O and filter. Do not use unless colorless.

28.082 DETERMINATION

Adjust sample to 4 g/100 ml acidity as HOAc. Steam distill 50 ml adjusted sample and collect 50 ml distillate. Regulate distn so that ca 45 ml remains in distg flask when 50 ml distillate has been collected. All-glass app. is preferable; if not available, cover cork or rubber stoppers with Sn or Al foil. App. illustrated in Fig. 31, **18.015**(**a**), is convenient. Keep distillate and reagents at 25°.

Transfer the 50 ml distillate to 500 ml g-s. erlenmeyer. Add 10 ml H_2SO_4 (1+1) and 25 ml of the $KMnO_4$ soln. Accurately measure the $KMnO_4$ soln, draining pipet definite time. Hold at 25°, preferably in H_2O bath, exactly 1 hr. Then immediately add 20 ml of the KI soln and mix well. Titr. liberated I with the 0.5*N* $Na_2S_2O_3$.

Conduct blank detn at same time, using 50 ml H_2O, 10 ml H_2SO_4 (1+1), and 25 ml of the $KMnO_4$ soln.

(Ml 0.5*N* $Na_2S_2O_3$ required by blank − quantity used in detn) ÷ 2 = permanganate oxidation number of vinegar. Report on basis of adjusted vinegar (4% acid).

If permanganate oxidation number is >15, repeat detn, using 25 ml of the adjusted vinegar +25 ml H_2O. Repeat this reduction by ½ until ml $KMnO_4$ soln used is <15. Calc. permanganate oxidation number to basis of 50 ml adjusted vinegar.

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29. Sugars and Sugar Products

(The compound referred to in this chapter as "basic $Pb(OAc)_2$" is the uniform mixt. with composition corresponding to $3Pb(OAc)_2.2PbO$.)

SUGARS, SIRUPS, AND MOLASSES

29.001 Preparation of Sample—Official

(**a**) *Solids* (*sugars, etc.*).—Grind, if necessary, and mix thoroly to secure uniform samples. Raw sugars should be mixed thoroly and in shortest possible time, either on glass plate with spatula, reducing lumps, when present, with glass or iron rolling pin; or in large, clean, dry mortar, using pestle to reduce lumps when present.

(**b**) *Semi-solids* (*massecuites, etc.*).—Weigh 50 g sample, dissolve crystals of sugar in min. quantity of H_2O, wash into 250 ml vol. flask, dil. to mark, and mix thoroly; or weigh 50 g sample and dil. with H_2O to 100 g. If insol. material remains, mix uniformly by shaking before taking aliquots or weighed portions for various detns.

(**c**) *Liquids* (*molasses, sirups, etc.*).—Mix materials thoroly. If crystals of sugar are present, dissolve them either by heating gently (avoiding loss of H_2O by evapn), or by weighing whole mass, then adding H_2O, heating until completely dissolved, and after cooling, reweighing. Calc. all results to wt original substance.

Color of Raw Cane Sugars (*1*)—First Action

29.002 REAGENT

Filter-aid.—Celite analytical filter-aid (Johns-Manville).

29.003 APPARATUS

Fractionator.—Construct fractionator of 35 mm i. d., heavy-wall Pyrex tubing, 145 mm long from top to bottom shoulder where it is sealed to ₮ stopcock with 3 mm bore and 9 mm o. d. tubing. Leave 55 mm stem below stopcock. Seal tube, 9 mm o. d., 45 mm long, to body of fractionator 45 mm below top. Connect to büchner and source of vac. thru "T" tube as shown in Fig. 61.

29.004 DETERMINATION

Place 60 g sample in flask, add 40 g boiling H_2O, and rotate flask until all sugar dissolves. Add 3 g Celite and shake mixt. vigorously 1 min. Assemble special filtration app., and with stopcock open, place paper, S&S No. 589 blue ribbon, 7 cm diam., in büchner, wet down with H_2O, and suck excess H_2O by vac. thru filtering tube into flask. Close stopcock and pour well-shaken mixt. of sugar soln and Celite evenly over paper. Filter at constant vac. of 24″. Collect ca 10 ml of first filtrate, which is somewhat turbid, in filtering tube and run into flask by opening stopcock. Close stopcock, collect another 10 ml filtrate in filtering tube, and run into flask as before to wash inner walls of tube free from any small particles of turbidity. Keep bed of Celite well covered with sugar soln during entire filtration. *Do not let it run dry.* Collect final clear filtrate in filtering tube, transfer to small bottle or to small, g-s. erlenmeyer, and mix thoroly. Det. refractometer Brix on portion of soln and calc. concn, *c* (g dry substance/ml soln), by

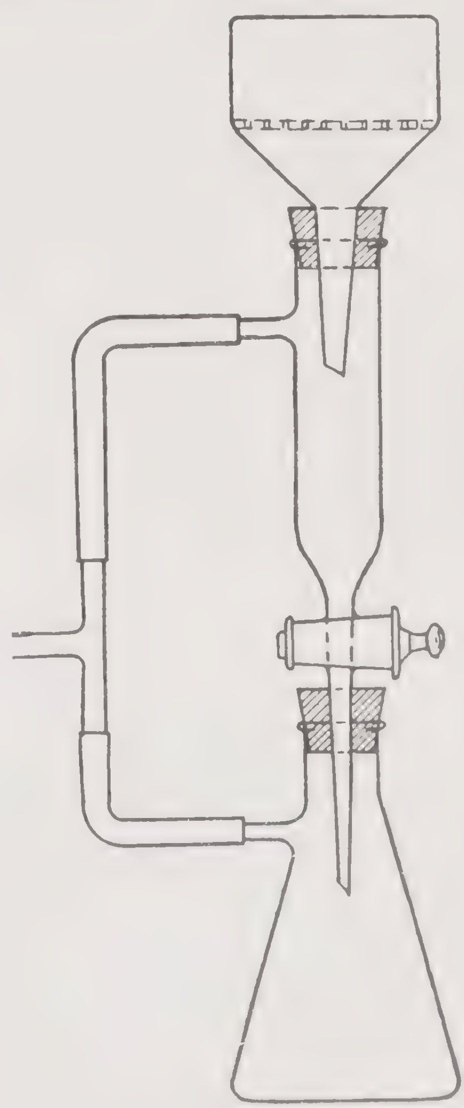

FIG. 61.—FRACTIONATOR

multiplying Brix by corresponding true density and dividing by 100.

Det. % transmittance, T, at 560 mμ on spectrophotometer with wavelength and transmittance scales that have been checked, preferably with std glass filter supplied by NBS. Use distd H_2O as the 100% T std. Cell thickness, b (in cm), used should be such that readings are within range 25–75% T.

Calc. attenuation index, $a_c^* = -(\log T)/bc$.

Moisture

(Applicable to both cane and beet, raw and refined sugars)

29.005 *Direct Drying—Procedure*

Dry ca 5 g prepd sample, **29.001**(a), in flat dish (Ni, Pt, or Al with tight-fit cover), 3 hr at 100°. Remove dish, cover, cool in desiccator, and weigh. Redry 1 hr and repeat process until change in wt between successive dryings at 1 hr intervals is not >2 mg. In case of large-grain sugars, increase temp. to 105–110° in final heating periods to expel last traces of occluded H_2O. Report loss in wt as H_2O.

29.006 *Vacuum Drying—Official*

(Applicable to both cane and beet, raw and refined sugars)

Dry 2–5 g prepd sample, **29.001**(a), in flat dish (Ni, Pt, or Al with tight-fit cover), 2 hr at not >70° (preferably 60°), under pressure not >50 mm Hg. Remove dish from oven, cover, cool in desiccator, and weigh. Redry 1 hr and repeat process until change in wt between successive dryings at 1 hr intervals is not >2 mg.

NOTE: Bleed oven with current of dry air during drying to insure removal of H_2O vapors.

29.007 *Drying upon Pumice Stone—Official*

(Applicable to massecuites, molasses, and other liquid and semi-liquid products)

Prep. pumice stone of 2 grades of fineness, one to pass thru 1 mm sieve, other thru 6 mm but not 1 mm sieve. Digest each 8 hr with H_2SO_4 (1+4) on steam bath. Wash acid-free and heat to dull redness. Make detn in flat metal dish 60 mm diam. Place 3 mm layer of the fine pumice stone on bottom of dish, then 6–10 mm layer of coarse pumice stone; dry and weigh. Dil. sample with weighed portion of H_2O so that dild material contains 20–30% solid matter. Weigh into dish, prepd as described, quantity of dild sample to yield ca 1 g dry matter. If this weighing cannot be made rapidly, use weighing bottle provided with cork thru which pipet passes. Dry at 70° under pressure not >50 mm Hg, making trial weighings at 2 hr intervals toward end of drying period until change in wt is not >2 mg. Report loss in wt as H_2O. For substances contg little or no levulose or other readily decomposable substance, drying may be made in oven at 100°. (*See* NOTE, **29.006**.)

29.008 *Drying upon Quartz Sand (2)—Official*

(Applicable to massecuites, molasses, and other liquid and semi-liquid products)

Digest pure quartz sand that passes No. 40 but not No. 60 sieve with HCl, wash acid-free, dry, and ignite. Preserve in stoppered bottle. Place 25–30 g prepd sand and short stirring rod in dish ca 55 mm diam., and 40 mm deep, fitted with cover. Dry thoroly, cover dish, cool in desiccator, and weigh immediately. Add enough dild sample of known wt to yield ca 1 g dry matter and mix thoroly with sand. Heat on steam bath 15–20 min., stirring at 2–3 min. intervals, or until mass becomes too stiff to manipulate readily. Dry at <70° (preferably 60°) under pressure not >50 mm Hg, making trial weighings at 2 hr intervals toward end of drying period (ca 18 hr) until change in wt is not >2 mg.

For materials contg no levulose or other readily decomposable substance, dry 8–10 hr at atmospheric pressure in oven at 100°, cool in desiccator, and weigh, repeating heating and weighing until loss in 1 hr heating is not >2 mg. Report loss in wt as H_2O.

As dry sand, as well as dried sample, absorbs appreciable quantity of moisture on standing over most desiccating agents, make all weighings as quickly as possible after cooling in desiccator. (*See* NOTE, **29.006**.)

Solids

29.009 *By Means of Spindle—Official*

(Accurate only when applied to pure sucrose solns, but extensively used for approx. results with liquid sugar products contg invert sugar and other non-sucrose solids.)

(a) *Direct.*—Density of juices, sirups, etc., is conveniently detd with Brix or Baumé hydrometer, preferably former as scale graduations agree closely with % total solids. Table for comparison of degrees Brix (% by wt of pure sucrose in pure solns), degrees Baumé (modulus 145), sp. gr. at 20/4°, and sp. gr. at 20/20° is given in **43.003**.

Use spindle graduated in tenths and as limited as possible in range of degrees recorded, and cylinder of sufficient diam. to permit spindle to come to rest without touching sides. Let soln come as nearly as practicable to same temp. as air at time of reading, and if this varies >1° from temp. at which spindle was graduated (20°), apply correction according to **43.004**. Before taking reading, let soln stand in cylinder until all air bubbles escape and all fatty or waxy materials come to top and are skimmed off. (Air bubbles

may be conveniently removed by applying vacuum to cylinder by means of tube passing thru stopper inserted in top of cylinder.)

(b) *Double dilution.*—If sample is too dense to det. density directly, dil. weighed portion with weighed quantity of H_2O, or dissolve weighed portion and dil. to known vol. with H_2O. In first instance, % total solids is calcd by following formula:

% solids in undild material $= WS/w$, in which $S = \%$ solids in dild material; $W =$ wt dild material; and $w =$ wt sample taken for diln.

When diln is made to definite vol., use following formula:

% solids in undild material $= VDS/w$, where $V =$ vol. dild soln at given temp; $D =$ sp. gr. of dild soln at same temp.; $S = \%$ solids in dild soln at same temp.; and $w =$ wt sample taken for diln.

Calcn is simplified by mixing equal wts sugar product and H_2O, and multiplying Brix of soln by 2.

29.010 *By Means of Pycnometer (3)—Official*

(a) *Specific gravity (in vacuo or in air).*—Det. sp. gr. of soln at 20/4°, 20/20° *in vacuo*, or 20/20° in air as in **9.011**, using either pycnometers described in **9.009(b)** or other suitable type. Det. % by wt of solids as sucrose from appropriate table, **43.003** or **43.005.** When density of substance is too high for direct detn, dil. and then calc. sucrose content of original material as in **29.009(b)**.

(b) *Specific gravity of molasses.*—Use special calibrated 100 ml vol. flask with neck ca 8 mm i. d. Weigh empty flask and then fill with molasses, using long-stem funnel reaching below graduation mark, until level of molasses is up to lower end of neck of flask. (Flow of molasses may be stopped by inserting glass rod of suitable size into funnel so as to close stem opening.) Remove funnel carefully to prevent molasses from coming in contact with neck, and weigh flask and molasses. Add H_2O almost to graduation mark, running it down side of neck to prevent mixing with molasses. Let stand several hr or overnight for bubbles to escape. Place flask in constant temp. H_2O bath, preferably at 20°, and leave until it reaches temp. of bath; then dil. to vol. at that temp. with H_2O. Weigh. Reduce wt molasses to *in vacuo* and calc. density. Ascertain corresponding Brix or Baumé reading from **43.003.**

Example:	*grams*
A, wt H_2O content of flask at 20° *in vacuo*	= 99.823
B, wt molasses at 20° *in vacuo*	=132.834
C, wt molasses and H_2O at 20° *in vacuo*	=137.968
$A-(C-B)=$ wt H_2O occupying space of molasses *in vacuo*	= 94.689

$$\frac{132.834}{94.689} = 1.403 \text{ sp. gr. } \left(\frac{20°}{20°}\right) \text{ molasses.}$$

29.011 *By Means of Refractometer (4)—Official*

(Applicable only to liquid samples contg no undissolved solids)

Det. refractometer reading of soln at 20° and obtain corresponding % dry substance from either direct reading, if sugar refractometer is used, or from **43.007,** if instrument gives readings in terms of refractive index. Circulate H_2O at constant temp., preferably 20°, thru jackets of refractometer or thru trough of immersion instrument, long enough to let temp. of prisms and of sample reach equilibrium, continuing circulation during observations and taking care that temp. is held constant.

If detn is made at temp. other than 20°, or if humidity causes condensation of moisture on exposed faces of prisms, make measurements at room temp. and correct readings to std temp. of 20° from **43.008.** If soln is too dark to be read in instrument, dil. with concd sugar soln; never use H_2O for this purpose. Mix weighed quantities of soln under examination and soln of pure sugar of about same strength, and obtain quantity of dry substance in former by following formula: $x=[(A+B)C-BD]/A$, in which $x = \%$ dry substance to be found; $A =$ wt (g) sample mixed with B; $B =$ wt (g) sugar soln used in diln; $C = \%$ dry substance in mixt. $A+B$ obtained from refractive index; and $D = \%$ dry substance in pure sugar soln obtained from its refractive index.

For liquid products contg invert sugar, correct % solids obtained from **43.007** by adding 0.022 for each % invert sugar in sample.

Ash—Official

29.012 *Method I.*

Heat sample of appropriate wt for product being examined (usually 5–10 g) in 50–100 ml Pt dish at 100° until H_2O is expelled; add few drops pure olive oil and heat slowly over flame until swelling stops. Place dish in muffle at ca 525° and leave until white ash is obtained. Moisten ash with H_2O, dry on steam bath and then on hot plate, and re-ash in muffle at 525° to constant wt.

29.013 *Method II.*

Carbonize sample of appropriate wt for product being examined (usually 5–10 g) in 50–100 ml Pt dish at ca 525° and treat charred mass with hot H_2O to dissolve sol. salts. (In case of low-purity products, addn of few drops pure olive oil, as in **29.012,** may be desirable.) Filter thru ashless paper, ignite paper and residue to white ash, add

filtrate of sol. salts, evap. to dryness, and ignite at ca 525° to constant wt.

29.014 *Sulfated Ash—Official*

Weigh 5 g sample into 50–100 ml Pt dish, add 5 ml 10% (by wt) H_2SO_4, ignite until sample is well carbonized, and then ash in muffle at ca 550°. Cool, add 2–3 ml 10% H_2SO_4, evap. on steam bath, dry on hot plate, and again ignite at 550° to constant wt. Express result as % sulfated ash.

29.015 Soluble and Insoluble Ash—Official

Ash sample as in **29.012** or **29.013**. Add H_2O to ash in the Pt dish, heat nearly to boiling, filter thru ashless paper, and wash with hot H_2O until combined filtrate and washings measure ca 60 ml. Return paper and contents to Pt dish, ignite carefully, cool, and weigh. Calc. % H_2O-sol. and H_2O-insol. ash.

29.016 Alkalinity of Soluble Ash—Official

Cool filtrate from **29.015** and titr. with 0.1*N* HCl, **42.009–42.010,** using Me orange, **4.004**(g). Express alky in terms of ml 1*N* acid/100 g sample.

29.017 Alkalinity of Insoluble Ash—Official

Add excess of 0.1*N* HCl (usually 10–15 ml) to ignited insol. ash in Pt dish, **29.015,** heat to incipient boiling on asbestos plate, cool, and titr. excess HCl with 0.1*N* NaOH, using Me orange. Express alky in terms of ml 1*N* acid/100 g sample.

29.018 Mineral Adulterants in Ash (*5*)—**First Action**

In large porcelain evapg dish, mix 100 g sample with ca 35 g H_2SO_4 and evap. to sirupy consistency. Pass elec. current thru it while stirring by placing one Pt electrode in bottom of dish near one side and attaching other to lower end of glass rod with which contents are stirred. Begin with current of ca 1 ampere and gradually increase to 4. In 10–15 min. mass is reduced to fine, dry char that may be readily burned to white ash in original dish over free flame or in muffle.

Note: This method is preferred to ordinary method of heating with H_2SO_4, especially in case of molasses, because, if properly manipulated, material comes quickly into form of very finely divided char or powder that is especially adapted for subsequent quick ignition.

If elec. current is not available, treat 100 g sample in large porcelain dish, evap. to sirupy consistency with enough H_2SO_4 to carbonize mass thoroly, and ignite in usual manner.

Following adulterants may be present: salts of Sn, used in molasses to bleach; mineral pigments, such as $PbCrO_4$ in yellow confectionery; oxides of Fe, sometimes used to simulate color of chocolate; and Cu. These elements may be detected by usual qualitative tests.

29.019 Nitrogen—Official

Det. N in 5 g sample as in **2.036,** using larger quantity of the H_2SO_4 if necessary for complete digestion.

Sucrose—Polarimetric Methods

General Procedure

Rules of International Commission for Uniform Methods of Sugar Analysis (*6*)

29.020 (**a**) *Standardization of Saccharimeter Scale—Official*

Saccharimeter scale must be graduated in conformity with International Sugar Scale adopted by International Commission for Uniform Methods of Sugar Analysis. Rotations on this scale are designated as degrees sugar (°S).

Basis of calibration of 100° point on International Sugar Scale is polarization of normal soln of pure sucrose (26.000 g/100 ml) at 20° in 200 mm tube, using white light and dichromate filter defined by Commission (**b**). This soln, polarized in room or cabinet, temp. of which is also 20°, must give saccharimeter reading of exactly 100°S. Temp. of sugar soln during polarization must be kept constant at 20°.

Following rotations hold for normal quartz plate of International Sugar Scale: Normal Quartz Plate = 100°S = 40.690° ± 0.002° (λ = 5461 Å) at 20°

$$1° (\lambda = 5461 \text{ Å}) = 2.4576°S$$

Normal Quartz Plate = 100°S = 34.620° ± 0.002° (λ = 5892.5Å) at 20°

$$1° (\lambda = 5892.5 \text{ Å}) = 2.8885°S$$

According to detn of Bates and Jackson (*7*) at NBS, Herzfeld-Schönrock scale reading for 26 g pure dry sucrose under above conditions is 99.89_5 °S. Scale reading has been redetd by Balch and Hill (*8*) and by Zerban, Gamble, and Hardin (*8*), who found values of 99.907° and 99.912°, resp. Av. value of these independent investigations is 99.90_5. Value 99.90° was adopted by International Commission in 1932.

For existing instruments graduated on Herzfeld-Schönrock scale, it is permitted either to change saccharimeter scale or to use wt *w* of 26.026 g in 100 ml.

(**b**) *Directions for Raw Sugars—Official*

In general, make all polarizations at 20°. For countries where mean temp. is >20°, saccharimeters may be adjusted at 30° or any other suitable

temp., under conditions specified above, provided sugar soln is dild to final vol. and polarized at this same temp.

In detg polarization of substances contg sugar, employ only half-shade instruments, either single or double wedge, and either 200 mm or 400 mm instruments. During observation keep app. in fixed position and so far removed from source of light that polarizing nicol is not warmed. As sources of light, employ lamps that give strong white illumination or Na lamp. Whenever there is any irregularity in source of light, place thin ground-glass plate between source of light and polariscope so as to render illumination uniform.

Before and after each set of observations, chemist must ascertain correct adjustment of his saccharimeter, using stdzd quartz plates. He must previously ascertain accuracy of wts, polarization flasks, observation tubes, and cover glasses. (Scratched cover glasses must not be used.) Make several readings and take mean thereof but do not reject any reading.

Quartz plates are stdzd to second decimal place, and by their use, quick and accurate test can be made. In using such plates for testing saccharimeters, it is necessary that instrument and plate be at same temp. (preferably 20°) when observation is made. Different points of scale, preferably 20°, 50°, 80°, and 100°S, should be tested against the plates. Scale may also be stzd by means of carefully calibrated telescopic control tube (*9*). Telescopic control tube of high accuracy has been described by Zerban, Gamble, and Hardin (*8*).

In detg polarization, use whole normal wt for 100 ml or multiple for any corresponding vol. Bring soln exactly to mark at proper temp. and after wiping out neck of flask with filter paper, add min. quantity of *dry basic* $Pb(OAc)_2$ (Horne's dry $Pb(OAc)_2$, **29.021**(**c**)), shake to dissolve, and pour all clarified sugar soln on rapid, air-dry filter. Cover funnel at start of filtration. Reject first 25 ml filtrate and use remainder (must be perfectly clear) for polarization. In no case return whole soln or any part to filter. If filtrate is cloudy after 25 ml has been rejected, begin new detn. Polarize in 200 mm tube. If, after all means have been used to effect proper decolorization, soln is too dark to read, use 100 mm tube and multiply reading by 2.

Other permissible clarifying and decolorizing agents are alumina cream, **29.021**(**b**), or concd alum soln. Do not use boneblack or decolorizing powders.

Whenever white light is used in polarimetric detns, it must be filtered thru soln of $K_2Cr_2O_7$ of such concn that % $K_2Cr_2O_7 \times$ length of column of soln in cm = 9. Double this concn in polarizing carbohydrate materials of high rotation dispersion, such as commercial glucose, etc.

(**c**) *Normal Weights and Conversion Factors of Different Saccharimeter Scales*

(1) *Herzfeld-Schönrock Scale.*—Normal wt = 26.026 g/100 ml soln. 1° = 0.34657° Angular Rotation D*.

(2) *International Sugar Scale.*—Normal wt = 26.000 g/100 ml soln. 1° = 0.34620° Angular Rotation D*.

(3) *French Sugar Scale.*—Normal wt = 16.269 g/100 ml soln. 1° = 0.21667° Angular Rotation D*.

29.021 *Preparation and Use of Clarifying Reagents (10)—Official*

(**a**) *Basic lead acetate soln.*—Activate litharge by heating 2.5–3 hr at 650–670° in muffle (cooled product should be lemon color). Boil 430 g neutral $Pb(OAc)_2.3H_2O$, 130 g freshly activated litharge, and 1 L H_2O 30 min. Let mixt. cool and settle; then dil. supernatant to sp. gr. of 1.25 with recently boiled H_2O. (Solid basic $Pb(OAc)_2$ may be substituted for the normal salt and litharge in prepn of soln. Because of error caused by vol. of ppt this reagent is not recommended for clarifying products of low purity.)

(**b**) *Alumina cream.*—Prep. cold satd soln of alum in H_2O. Add NH_4OH with constant stirring until soln is alk. to litmus, let ppt settle, and wash by decantation with H_2O until wash H_2O gives only slight test for sulfates with $BaCl_2$ soln. Pour off excess H_2O and store residual cream in stoppered bottle. (Alumina cream is suitable for clarifying light-colored sugar products or as adjunct to other agents when sugars are detd by polariscopic or reducing sugar methods.)

(**c**) *Dry basic lead acetate.*—Conforms to ACS specifications. Of this salt, ca $\frac{1}{3}$ g = 1 ml basic $Pb(OAc)_2$ soln, (**a**). In making clarification, add small quantity of dry salt to sugar soln after dilg to vol., and shake; then add more salt and shake again, repeating addn until pptn is complete, but avoiding any excess. When molasses or any other substance producing heavy ppt is being clarified, add some dry, coarse sand to break up pellets of basic $Pb(OAc)_2$ and ppt. (Unless in excess, dry basic $Pb(OAc)_2$ does not cause vol. error.)

(**d**) *Neutral lead acetate soln.*—Prep. satd soln of neutral $Pb(OAc)_2$ and add to sugar soln before dilg to vol. (This reagent may be used for clarifying light-colored sugar products when sugars are detd by polariscopic methods, and its use is imperative when reducing sugars are detd in soln used for polarization.)

To remove excess Pb used in clarification, add to clarified filtrate anhyd. K or Na oxalate in small quantities until test for Pb in filtrate is negative; then refilter.

* Designation D refers to Na light of 5892.5 Å.

29.022 *Temperature Corrections for Polarization of Sugars (11)—First Action*

(a) *Refined sugars.*—Polarizations of sugars testing 99 or above, when made at temp. other than 20°, may be calcd to polarizations at 20° by following formula:

$P_{20}=p^t[1+0.0003\ (t-20)]$, where p^t=polarization at temp. read, and t=temp. at which polarization is read. (May be applied to beet sugar and raw cane sugars polarizing not <96°S without appreciable error.)

(b) *Raw sugars.*—Polarization of raw cane sugars <96°S when made at temps other than 20°, may be calcd to polarizations at 20° by following formula:

$P_{20}=p^t+0.0015\ (p^t-80)\ (t-20)$, where p^t and t are same as in (a).

When % levulose in the sugar is known (in case of honeys and sugar cane products = ca ½ reducing sugars), use following formula:

$P_{20}=p^t+0.0003S\ (t-20)-0.00812L\ (t-20)$, where p^t and t are same as in (a); S = % sucrose; and L = % levulose.

These formulas give results agreeing closely with polarizations obtained at 20° if sugar is of av. normal composition.

29.023 *Mutarotation—Procedure*

Products, such as honey and commercial glucose, that contain dextrose or other reducing sugars in cryst. form or in soln at high density may show mutarotation under conditions prevailing during analysis. Only constant rotation should be used in polarimetric methods. To obtain this, let soln prepd for polarization stand overnight before making reading. If it is desired to make reading immediately, heat neutral soln (pH ca 7.0) to boiling or add few drops NH_4OH, before dilg to vol.; or, if soln has been made to vol., add dry Na_2CO_3 until just distinctly alk. to litmus paper. (Do not let slightly alk. solns stand at such high temps or for such lengths of time as to cause destruction of levulose.) Det. completion of mutarotation by making readings at 15–30 min. intervals until constant.

Sucrose in Absence of Raffinose

By Polarization Before and After Inversion with Invertase (12)—Official

29.024 REAGENT

Invertase soln.—Commercial invertase prepns are available on market. If it is desired to prep. soln in laboratory, method described in (1) may be used. In either case, prepn may be further purified and concd by ultrafiltration method described in (3). Commercial prepns may also be purified by dialysis and then reconcd by evapg *in vacuo* at temp. not >40°.

(1) *Crude invertase soln.*—Mix yeast with H_2O in proportion of 10 lbs compressed bakers' yeast to 5 L H_2O. Add 2 L toluene and stir thoroly at frequent intervals during first 24 hr. Let stand 7 days with occasional stirring, and filter by gravity thru large fluted papers. Mix residue with 2 L H_2O, filter, and combine filtrates. Purify (*13*) by adding 15 g neutral $Pb(OAc)_2.3H_2O$ to each L of ext. and filtering on paper after all $Pb(OAc)_2$ dissolves. Complete purification immediately by dialysis or by washing on ultrafilter as in (3).

(2) *Collodion ultrafilter* (*14*).—Dissolve 6 g sol. (in alcohol and ether mixt.) pyroxylin or nitrocellulose in mixt. of 50 ml absolute alcohol and 50 ml absolute ether by adding the alcohol to the cotton, letting mixt. stand in stoppered flask 10 min., adding the ether, and shaking. Let soln stand overnight. Pour ca 100 ml into 2 L cylinder, and coat entire inside surface of cylinder with the collodion. Drain, and dry 10 min. Fill cylinder with H_2O, let stand 10–15 min., pour out the H_2O, and remove collodion sack. Test for leaks by filling with H_2O. Slit open longitudinally and cut out circular piece 7–8″ diam. Cut bottom from 2 L bottle or erlenmeyer and grind edge smooth. Place it upon still moist collodion disk, fold edge of disk up around bottle, and cement it thereto with collodion that contains increased proportion of ether. Place 3 or 4 thicknesses of wet filter paper in 8″ büchner. Place bottle with collodion membrane upon filter paper. Pour melted Vaseline, to depth of 1″, between bottle and inside of funnel. Provide bottle with small mechanical stirring device.

(3) *Washing and concentrating of invertase soln by ultrafiltration.*—Filter 4 L partially purified soln thru the ultrafilter, stirring continuously, until ca 1 L remains. Wash with distd H_2O added from constant level device until filtrate is colorless (3 or 4 L of wash H_2O is required). Discard filtrate and transfer invertase soln to stoppered bottle. During entire process and in storage preserve invertase soln with toluene.

(4) *Activity of invertase soln.*—Following test for activity of invertase soln is usually adequate: Dil. 1 ml invertase prepn to 200 ml. Transfer 10 g sucrose (granulated sugar) to sugar flask graduated at 100 and 110 ml, dissolve in ca 76 ml H_2O, add 2 drops HOAc, and dil. to 100 ml mark. To the 100 ml sugar soln add 10 ml of the dil. invertase soln and mix thoroly and rapidly, noting exact time at which solns are mixed. After exactly 60 min. make portions of soln just distinctly alk. to litmus paper with anhyd. Na_2CO_3 and polarize in 200 mm tube at 20°. If invertase soln is sufficiently active, the alk. soln will polarize ca 31°S without correcting for diln to 110 ml and optical activity of invertase soln.

If more exact information concerning activity of invertase prepn is desired, det. its velocity

constant as follows: Dil. 1 ml of the invertase soln to 200 ml at 20°; place in constant temp. bath at 20°; and when soln reaches this temp., pipet 20 ml into flask contg 200 ml sucrose soln (10 g/100 ml concn) previously made distinctly acid to Me red (corresponding to pH ca 4.6) by addn of HOAc and also brought to temp. of 20° in same bath. Mix thoroly and promptly, and note time at which invertase soln was added. Keep sucrose-invertase mixt. in constant temp. bath; remove portions after 15, 30, and 45 min.; make each portion just distinctly alk. to litmus paper with anhyd. Na_2CO_3 immediately after removing; and polarize at 20°. Correct all polarizations for polarization of invertase soln. Calc. velocity constant, k, for each of polarizations (at time t) subsequent to initial polarization by following formula:

$$k = [\log_{10} 1.32\, R_0 - \log_{10} (R_t + 0.32\, R_0)]/t,$$

where k = unimolecular reaction velocity constant; t = number of min. elapsing from time invertase and sucrose solns were mixed until inversion was stopped by addn of Na_2CO_3; R_0 = initial polarization (calcd by multiplying polarization of sucrose soln by 10/11 and correcting for polarization of invertase soln); and R_t = polarization at time t.

Invertase soln of sufficient activity (*15*) should yield av. value for k (for various time periods) of at least 0.1, after multiplying k value directly obtained by 200 in order to correct for initial diln of invertase soln. Diln of invertase soln mentioned above is made solely for purpose of detg its activity; original, undild invertase soln is used as inverting reagent in detn of sucrose, **29.025**, unless activity of original invertase soln greatly exceeds k value of 0.1, and it is desirable to conserve the invertase. In this case, dil. to k value of 0.1, which is done in same manner as dilg other solns to std strength. Activity of invertase prepn required for rapid inversion, **29.025(c)**, is same as that needed for overnight inversion, **29.025(b)**, but proportion of invertase prepn used in former case is twice that used in latter instance.

29.025 DETERMINATION

(**a**) *Direct reading.*—Dissolve double normal wt of sample (52 g), or fraction thereof, in H_2O in 200 ml vol. flask; add necessary clarifying agent, **29.021(a)**, (**b**), or (**d**), avoiding any excess; shake, dil. to mark with H_2O, mix well, and filter, keeping funnel covered with watch glass. Reject first 25 ml filtrate.

If Pb clarifying agent was used, remove excess Pb from soln when enough filtrate collects by adding anhyd. Na_2CO_3, little at time, avoiding any excess; mix well and filter again, rejecting first 25 ml filtrate. (Instead of weighing 52 g into 200 ml flask, two 26 g portions may be dild to 100 ml each, and treated exactly as described. Depending on color of product, multiples or fractions of normal wt may be used, and results reduced by calcn to basis of 26 g/100 ml.)

Pipet one 50 ml portion Pb-free filtrate into 100 ml flask, dil. with H_2O to mark, mix well, and polarize in 200 mm tube. Result, multiplied by 2, is direct reading (P of formula given below) or polarization before inversion. (If 400 mm tube is used, reading equals P.) If there is possibility of mutarotation, proceed in as **29.023.**

(**b**) *Invert reading.*—First det. quantity of HOAc necessary to make 50 ml of the Pb-free filtrate distinctly acid to Me red; then to another 50 ml of the Pb-free soln in 100 ml vol. flask add requisite quantity of acid and 5 ml of the invertase soln, fill flask with H_2O nearly to 100 ml, and let stand overnight (preferably at not <20°).

Cool, and dil. to 100 ml at 20°. Mix well and polarize at 20° in 200 mm tube. If in doubt as to completion of hydrolysis, let portion of soln remain several hr and again polarize. If there is no change from previous reading, inversion is complete. Carefully note reading and temp. of soln. If it is necessary to work at temp. other than 20°, which is permissible within narrow limits, complete vols and make both direct and invert readings at same temp. Correct polarization for optical activity of invertase soln and multiply by 2. Calc. % sucrose by following formula:

$$S = \frac{100(P - I)}{132.1 + 0.0833(m - 13) - 0.53(t - 20)},$$

where S = % sucrose; P = direct reading, normal soln; I = invert reading, normal soln; t = temp. at which readings are made; and m = g total solids in 100 ml inverted soln (total solids in 50 ml original soln). For liquids, det. total solids in original sample as % by wt, as in **29.011**, and multiply this figure by wt original sample in 100 ml invert soln.

(**c**) *Rapid inversion at 55–60°* (*16*).—If more rapid inversion is desired, proceed as follows: Prep. sample as in (**a**) and to 50 ml Pb-free filtrate in 100 ml vol. flask add enough HOAc to render soln distinctly acid to Me red, **29.024**(4). Det. quantity of HOAc required before pipetting the 50 ml portion as in (**b**). Add 10 ml invertase soln, mix thoroly, place flask in H_2O bath at 55–60°, and let stand at that temp. 15 min., shaking occasionally.

Cool, add Na_2CO_3 until distinctly alk. to litmus paper, dil. to 100 ml at 20°, mix well, and det. polarization at 20° in 200 mm tube. Let soln remain in tube 10 min. and again det. polarization. If there is no change from previous reading, mutarotation is complete. Carefully note reading and temp. of soln. Correct polarization for optical activity of the invertase soln and multiply by 2. Calc. % sucrose by formula given in (**b**).

If soln has been made so alk. as to cause destruction of sugar, polarization, if negative, will in general decrease, since decomposition of levulose ordinarily is more rapid than that of other sugars present. If soln has not been made alk. enough to complete mutarotation quickly, polarization, if negative, will in general increase. As analyst gains experience he may omit polarization after 10 min. if he has satisfied himself that he is adding enough Na_2CO_3 to complete mutarotation at once without causing any destruction of sugar during period intervening before polarization.

29.026 *By Polarization Before and After Inversion with Hydrochloric Acid (17)—Official*

(a) *Direct reading.*—Prep soln as in **29.025(a)**. Pipet 50 ml Pb-free filtrate into 100 ml vol. flask; add 2.315 g NaCl and 25 ml H_2O. Dil. to mark with H_2O at 20° and polarize in 200 mm tube at 20°. Multiply reading by 2 to obtain direct reading.

(b) *Invert reading.*—Pipet 50 ml portion Pb-free filtrate into 100 ml vol. flask and add 20 ml H_2O. Add, little by little, while rotating flask, 10 ml HCl (sp. gr. 1.1029 at 20/4° or 24.85° Brix at 20°). Heat H_2O bath and adjust burner to keep bath at 60°. Place flask in H_2O bath, agitate continuously ca 3 min., and leave flask in bath exactly 7 min. longer. Plunge flask at once into H_2O at 20°.

When contents cool to ca 35°, dil. almost to mark. Leave flask in bath at 20° at least 30 min. longer and finally dil. to mark. Mix well and polarize soln in 200 mm tube provided with lateral branch and H_2O jacket, keeping temp. at 20°. This reading must also be multiplied by 2 to obtain invert reading. If it is necessary to work at temp. other than 20°, which is permissible within narrow limits, vols must be completed and both direct and invert polarizations must be made at exactly same temp.

Calc. sucrose by following formula:

$$S = \frac{100(P - I)}{132.56 + 0.0794(m - 13) - 0.53(t - 20)},$$

where $S = \%$ sucrose; $P =$ direct reading, normal soln; $I =$ invert reading, normal soln; $t =$ temp. at which readings are made; and $m =$ g total solids in 100 ml inverted soln (total solids in 50 ml original soln). For liquids, det. total solids in original sample as % by wt as in **29.011**, and multiply this figure by wt original sample in 100 ml invert soln.

(c) *Inversion at room temperature.*—Inversion may also be accomplished as follows: (1) Pipet 50 ml Pb-free filtrate into 100 ml vol. flask, add 20 ml H_2O and 10 ml HCl (sp. gr. 1.1029 at 20/4° or 24.85° Brix at 20°), and set aside 24 hr at not <22°; or (2) set aside 10 hr if >28°. Dil. to 100 ml at 20° and polarize as in (b). Under these conditions formula must be changed to following:

$$S = \frac{100(P - I)}{132.66 + 0.0794(m - 13) - 0.53(t - 20)}.$$

Sucrose and Raffinose *(18)*

By Polarization Before and After Treatment with Two Enzyme Preparations—Official

29.027 REAGENTS

(a) *Invertase soln (top yeast extract).*—Prep. as in **29.024.** This soln should be free from enzyme melibiase. Its invertase activity should be at least as great as that used for detn of sucrose in absence of raffinose, **29.024**(4).

(b) *Invertase-melibiase soln (bottom yeast extract).*—Prep. as in **29.024,** using bottom fermenting yeast (brewers' yeast) instead of bakers' yeast. Invertase activity should be at least as great as in (a).

Test melibiase activity of soln as follows: Add 2 ml soln to be tested to 20 ml weakly acid melibiose soln polarizing +20.0°S and let stand 30 min. at ca 20°. Add enough Na_2CO_3 to make soln slightly alk. to litmus paper. Prepn suitable for overnight hydrolysis of solns contg not >0.2 g raffinose in 100 ml should have hydrolyzed 35% of melibiose present under conditions mentioned; prepn suitable for overnight hydrolysis of solns contg not >0.65 g raffinose in 100 ml should have produced 50% hydrolysis of melibiose; and prepn suitable for overnight hydrolysis of solns contg 0.65–1.3 g raffinose in 100 ml should have hydrolyzed at least 70% of melibiose present under above conditions. Prepns of melibiose soln that polarize +20°S before hydrolysis, will polarize +16.4°, +14.9°, and +12.9°S after 35, 50, and 70% hydrolysis, resp.

29.028 DETERMINATION

With sugar beet products, weigh material specified in table, **29.029,** transfer to 300 ml vol. flask, add quantity of basic $Pb(OAc)_2$ soln indicated in table, and dil. to vol. at 20°. Mix thoroly and filter thru fluted paper in closely covered funnel, rejecting first 25 ml filtrate. When enough filtrate collects, remove Pb from soln by adding $NH_4H_2PO_4$ in as small excess as possible, **29.029.** This condition is readily detd after little practice by appearance of the $Pb_3(PO_4)_2$ ppt, which usually flocculates and settles rapidly in presence of slight excess of the salt. Mix well and filter, again rejecting at least first 25 ml filtrate.

Make direct polarization in 200 mm tube at 20° unless soln contains appreciable quantity of invert sugar, in which case pipet 50 ml portion Pb-free filtrate into 100 ml flask, dil. with H_2O to mark, mix well, and polarize at 20°, preferably in 400 mm tube. This reading, calcd to normal wt of 26 g in 100 ml and 200 mm tube length, is direct read-

ing (P) of formula given below for polarization before inversion.

Transfer two 50 ml portions of the Pb-free filtrate to 100 ml vol. flasks. To one add 5 ml invertase soln (top yeast ext.) and to other 5 ml invertase-melibiase soln (bottom yeast ext.), let stand overnight at room temp. (preferably not <20°), dil. to vol., mix well, and polarize at 20°, preferably in 400 mm jacketed tube.

If rapid hydrolysis is desired, add 10 ml of each of the enzyme solns to 50 ml portions Pb-free filtrate in 100 ml vol. flasks and place in H_2O bath 40 min. at 50–55°. Then add Na_2CO_3 until soln is slightly alk. to litmus paper, dil. to vol. at 20°, mix well, and polarize at 20°, preferably in 400 mm tube. Correct invert readings for optical activity of enzyme soln and calc. polarization to that of normal wt soln of 26 g/100 ml; also calc. reading to 200 mm tube length, if necessary.

Calc. % of anhyd. raffinose and sucrose from following formulas:

$$R = 1.354\,(A - B); \quad S = \frac{(P - 2.202A + 1.202B)100}{132.12 - 0.00718[132.12 - (P - 2.202A + 1.202B)]},$$

where $R = \%$ raffinose; $S = \%$ sucrose; $P =$ direct polarization, normal soln; $A =$ corrected polarization after top yeast hydrolysis, normal soln; and $B =$ corrected polarization after bottom yeast hydrolysis, normal soln.

Quantities A and B are treated algebraically.

29.029 *Quantities of sample and reagents required for clarification and deleading of beet sugar-house products*

MATERIAL	QUANTITY PER 100 ML	BASIC LEAD ACETATE (55° BRIX)	AMMONIUM DIHYDROGEN PHOSPHATE
	grams	*ml*	*gram*
Cossettes[a]	13	3	0.2
Pulp	100 ml[b]	2–4	0.2
Lime cake or sewer[c]	26.5	1.5	....[d]
Thin juice	52	2	0.2–0.3
Thick juice	26	4	0.3–0.4
White massecuite	13 or 26	3 or 6	0.3–0.7
High wash sirup	13 or 26	3 or 6	0.3–0.7
High green sirup	13 or 26	5 or 10	0.3–0.7
Raw or remelt massecuite	13	6	0.3–0.4
Raw or remelt sugar	26	3–4	0.3–0.4
Sugar melter	26	2–3	0.3–0.4
Low wash sirup	13	8–10	0.4–0.5
Low green sirup or molasses	13	10	0.4–0.5
Saccharate cakes and milk (carbonated)	26	4–6	0.3–0.4
Steffen waste and wash waters[c]	78 or 50 ml	2–3	0.2

[a] Usual method of extn, 26 g in 201.2 ml.
[b] Dil. to 110 ml.
[c] Neutralize with HOAc before adding basic $Pb(OAc)_2$.
[d] As Ca in soln will be partly pptd by the phosphate, it is necessary to add enough phosphate to complete the pptn of both Pb and Ca salts, and no definite quantity can be specified.

29.030 *By Polarization Before and After Inversion with Hydrochloric Acid - Official*

(Of value chiefly in analysis of beet products)

If direct reading is >1° higher than % sucrose as calcd by formula given in **29.026**(b), raffinose is probably present. Calc. sucrose and raffinose by following formulas (*19*):

When polarizations are made at 20°:

$S = (0.514P - I)/0.844$, and $R = (0.33P + I)/1.563$,

where $P =$ direct reading, normal soln; $I =$ invert reading, normal soln; $S = \%$ sucrose; and $R = \%$ anhyd. raffinose.

Following formulas (*19*) are applicable at all temps other than 20°:

$$S = \frac{P(0.478 + 0.0018t_2) - I(1.006 - 0.0003t_1)}{(0.908 - 0.0032t_2)(1.006 - 0.0003t_1)}$$

and

$$R = \frac{P(0.43 - 0.005t_2) + I(1.006 - 0.0003t_1)}{(1.681 - 0.0059t_2)(1.006 - 0.0003t_1)}$$

where $P =$ direct reading, normal soln; $I =$ invert reading, normal soln; $S = \%$ sucrose; $R = \%$ anhyd. raffinose; $t_1 =$ temp. of direct polarization; and $t_2 =$ temp. of invert polarization.

29.031 Sucrose by Double Dilution Method (*20*)—Official

(Substances in which vol. of combined insol. matter and ppt from clarifying agents is >1 ml from 26 g)

Weigh 13 g sample and dil. soln to 100 ml, using appropriate clarifier (basic $Pb(OAc)_2$ for dark-colored confectionery or molasses, and alumina cream for light-colored confectionery). Also weigh 26 g sample and dil. this second soln with the clarifier to 100 ml. Filter both solns, and obtain direct polariscopic readings. Invert each soln

as in **29.025(b)** or **(c)** or **29.026(b)** or **(c)** and obtain respective invert readings.

True direct polarization of sample = 4 times direct polarization of dild soln minus direct polarization of undild soln. True invert polarization = 4 times invert polarization of dild soln minus invert polarization of undild soln. Calc. sucrose from true polarizations thus obtained, using formula in **29.025(b)** or **29.026(b)** or **(c)** corresponding to method of inversion used.

Sucrose—Chemical Methods

29.032 *From Reducing Sugars Before and After Inversion—Official*

Det. reducing sugars as in **29.039** (clarification having been effected with neutral $Pb(OAc)_2$ as in **29.021(d)**) and calc. to invert sugar from **43.011** or **43.012**. Invert soln as in **29.025(b)** or **(c)**, or **29.026(b)** or **(c)**; exactly neutralize the acid; and again det. reducing sugars, but calc. them to invert sugar from tables referred to above, using invert sugar column alone. Deduct % invert sugar obtained before inversion from that obtained after inversion and multiply difference by 0.95 to obtain % sucrose. Dil. solns in both detns so that not >230 mg invert sugar is present in quantity taken for reduction. It is important that all Pb be removed from soln with anhyd. powd. K oxalate before reduction.

Commercial Glucose (Approximate) Polarimetric Methods—Procedure

29.033 *Substances Containing Little or No Invert Sugar*

Commercial glucose cannot be detd accurately, since quantities of dextrin, maltose, and dextrose present vary. However, in sirups in which quantity of invert sugar is too small to appreciably affect result, commercial glucose may be estimated approximately by following formula:

$$G = (a - S)100/211,$$

where G = % commercial glucose solids; a = direct polarization, normal soln; and S = % cane sugar.

Express results in terms of commercial glucose solids polarizing +211°S. (This result may be recalcd in terms of commercial glucose of any polarization desired.)

29.034 *Substances Containing Invert Sugar*

Prep. inverted half-normal soln of substance as in **29.026(b)**, except cool soln after inversion, make neutral to phthln with NaOH soln, slightly acidify with HCl (1+5), and treat with 5–10 ml alumina cream, **29.021(b)**, before dilg to mark. Filter, and polarize at 87° in 200 mm jacketed metal tube. Multiply reading by 200 and divide by factor 196 to obtain quantity of commercial glucose solids polarizing +211°S. (Result may be recalcd in terms of commercial glucose of any polarization desired.)

Invert Sugar—Chemical Methods

I. Lane-Eynon General Volumetric Method (21)—Official

29.035 REAGENTS

Soxhlet modification of Fehling soln.—Prep. by mixing equal vols of **(a)** and **(b)** immediately before use.

(a) *Copper sulfate soln.*—Dissolve 34.639 g $CuSO_4.5H_2O$ in H_2O, dil. to 500 ml, and filter thru glass wool or paper. Det. Cu content of soln (preferably by electrolysis, **29.045**) and so adjust that it contains 440.9 mg Cu/25 ml.

(b) *Alkaline tartrate soln.*—Dissolve 173 g Rochelle salt and 50 g NaOH in H_2O, dil. to 500 ml, let stand 2 days, and filter thru prepd asbestos, **29.038.**

(c) *Invert sugar std soln.*—To soln of 9.5 g pure sucrose, add 5 ml HCl and dil. with H_2O to ca 100 ml. Store several days at room temp. (ca 7 days at 12–15° or 3 days at 20–25°); then dil. to 1 L. (Acidified 1% invert sugar soln is stable for several months.) Neutralize aliquot with NaOH and dil. to desired concn immediately before use.

29.036 STANDARDIZATION AND TITRATION

Pipet accurately 10 or 25 ml mixed Soxhlet reagent, or 5 or 12.5 ml each of Soxhlet solns **29.035(a)** and **(b)**, into 300–400 ml flask. (Quantity of Cu taken differs slightly between two methods of pipetting, and method used must be consistent in stdzn and detn.) Prep. std soln of the pure sugar of such concn that >15 ml and <50 ml is required to reduce all the Cu; mg sugar required to completely reduce the Cu at different concns is given in **43.017** and **43.018.** Add sugar soln within 0.5–1.0 ml of total required, heat cold mixt. to boiling on wire gauze, and maintain moderate boiling 2 min. (coarse grains of C or other suitable inert material may be used to prevent bumping). Without removing flame add 1 ml *0.2% aq. methylene blue soln* and complete titrn within total boiling time of ca 3 min. by small addns. of sugar soln to decoloration of indicator. After complete reduction of Cu, methylene blue is reduced to colorless compound.

Multiply titer by mg/ml of the std soln to obtain total sugar required to reduce the Cu. Compare with tabulated value in **43.017** or **43.018** to det. correction, if any, to be applied to table. Small deviations from tabulated values may arise from variations in individual procedure or composition of reagents. If only approx. results (within 1%) are required, stdzn may be omitted,

provided specifications of analysis are rigidly observed.

29.037 DETERMINATION

If approx. concn of sugar in sample is unknown, proceed by incremental method of titrn. To 10 or 25 ml Soxhlet soln, add 15 ml of the sugar soln and heat to boiling over wire gauze. Boil ca 15 sec. and add rapidly further quantities of the sugar soln until only faintest perceptible blue color remains. Then add 1 ml 0.2% aq. methylene blue soln and complete titrn by adding the sugar soln dropwise. (Error resulting from this titrn will not generally be >1%.)

For higher precision repeat titrn, adding almost entire sugar soln required to reduce all Cu and proceed as above. In **43.017** or **43.018** find total reducing sugar corresponding to titer and apply correction previously detd. Calc. as follows: total reducing sugar required×100/titer = mg sugar in 100 ml.

II. Munson-Walker General Method (22)—Official

29.038 REAGENTS

Asbestos.—Digest asbestos, amphibole variety, with HCl (1+3) 2–3 days. Wash acid-free, digest similar period with 10% NaOH soln, and then treat few hr with hot alk. tartrate soln, **29.035(b)** (alk. tartrate solns that have stood for some time may be used for this purpose). Wash asbestos alkali-free; digest several hr with HNO_3 (1+3); and after washing acid-free, shake with H_2O into fine pulp. In prepg gooch, make film of the asbestos ¼″ thick and wash thoroly with H_2O to remove fine particles. If pptd Cu_2O is to be weighed as such, wash crucible with 10 ml alcohol and then with 10 ml ether; dry 30 min. at 100°, cool in desiccator, and weigh.

Other reagents and solns used are described in **29.035.** Solns may be clarified by neutral $Pb(OAc)_2$ soln, **29.021(d)** (never basic $Pb(OAc)_2$). Remove excess Pb with dry $Na_2C_2O_4$.

29.039 PRECIPITATION OF CUPROUS OXIDE

Transfer 25 ml of each of the $CuSO_4$ and alk. tartrate solns to 400 ml beaker of alkali-resistant glass and add 50 ml of the reducing sugar soln; or if smaller vol. of sugar soln is used, add H_2O to make final vol. 100 ml. Heat beaker on asbestos gauze over Bunsen burner, regulate flame so that boiling begins in 4 min., and continue boiling exactly 2 min. (It is important that these directions be strictly observed. To regulate burner for this purpose it is advisable to make preliminary tests, using 50 ml reagent and 50 ml H_2O, before proceeding with actual detn. Elec. heater may be used instead of burner.) Keep beaker covered with watch glass during heating.

Filter hot soln at once thru asbestos mat in porcelain gooch, using suction. Wash ppt of Cu_2O thoroly with H_2O at ca 60° and either weigh directly as Cu_2O, **29.040,** or det. quantity of reduced Cu by one of methods described in **29.041–29.045.** Conduct blank detn, using 50 ml of the reagent and 50 ml H_2O, and if wt Cu_2O obtained is >0.5 mg, correct result of reducing sugar detn accordingly. Alk. tartrate soln deteriorates on standing, and quantity of Cu_2O obtained in blank increases.

DETERMINATION OF REDUCED COPPER

29.040 *By Direct Weighing of Cuprous Oxide*

(Use only for detns in solns of reducing sugars of comparatively high purity. In products contg large quantities of mineral or org. impurities, including sucrose, det. the Cu in the Cu_2O by one of methods described in **29.041–29.045**, since the Cu_2O is likely to be contaminated with foreign matter.)

Prep. gooch as in **29.038.** Collect pptd Cu_2O on mat as in **29.039;** wash thoroly with hot H_2O, then with 10 ml alcohol, and finally with 10 ml ether. Dry ppt 30 min. in oven at 100°, cool, and weigh. Obtain from **43.011** wt invert sugar equiv. to wt Cu_2O.

Number of mg Cu_2O reduced by given quantity of reducing sugar varies, depending upon whether or not sucrose is present. In the table, absence of sucrose is assumed except in entries under invert sugar, where, in addn to column for invert sugar alone, one column is given for mixts of invert sugar and sucrose contg 0.4 g total sugar in 50 ml soln and one column for invert sugar and sucrose when the 50 ml soln contains 2 g total sugar. Two entries are also given under lactose and sucrose mixts, showing proportions of 1 part lactose to 4 and 12 parts sucrose, resp.

By Titration with Sodium Thiosulfate (23)

29.041 REAGENT

Thiosulfate std soln.—Prep. soln contg 39 g $Na_2S_2O_3.5H_2O$/L. Weigh accurately 0.2–0.4 g pure electrolytic Cu and transfer to 250 ml erlenmeyer roughly marked at 20 ml intervals. Dissolve Cu in 5 ml HNO_3 (1+1), dil. to 20 or 30 ml, boil to expel red fumes, add slight excess satd Br-H_2O, and boil until Br is completely removed. Cool, and add 10 ml NaOAc soln (574 g trihydrate/L). Prep. 12 g/100 ml KI soln made very slightly alk. to avoid formation and oxidation of HI. Add 10 ml of the KI soln and titr. with the $Na_2S_2O_3$ soln to light yellow. Add enough starch indicator, **4.004(f)**, to produce marked blue. As end point nears, add 2 g KCNS (24) and stir until completely dissolved. Continue titrn until ppt is perfectly white. 1 ml $Na_2S_2O_3$ soln = ca 10 mg Cu.

It is essential for $Na_2S_2O_3$ titrn that concn of KI in soln be carefully regulated. If soln contains <320 mg Cu, at completion of titrn 4.2–5 g KI should have been added for each 100 ml total soln. If greater quantities of Cu are present, add KI soln slowly from buret with constant agitation in quantities proportionately greater.

29.042 DETERMINATION

Wash pptd Cu_2O, cover gooch with watch glass, and dissolve the Cu_2O with 5 ml HNO_3 (1+1) directed under watch glass with pipet. Collect filtrate in 250 ml erlenmeyer roughly marked at 20 ml intervals, and wash watch glass and gooch Cu-free. Proceed as in **29.041,** beginning, "boil to expel red fumes . . ." Obtain wt reducing sugar equiv. to wt Cu from **43.012.**

By Titration with Potassium Permanganate (25)

29.043 REAGENTS

(**a**) *Potassium permanganate std soln.*—Approx. $0.1573N$, and contg 4.98 g/L. After aging several days, filter thru asbestos or fritted glass. Stdze by one of following methods:

(1) Transfer 0.35 g $Na_2C_2O_4$ (dried at 103°) to 600 ml beaker. Add 250 ml H_2SO_4 (5+95) previously boiled 10 min. and cooled to $27 \pm 3°$. Stir until $Na_2C_2O_4$ dissolves. Add 29–30 ml of the $KMnO_4$ soln at rate of 25–35 ml/min. while stirring slowly. Let mixt. stand until pink disappears (ca 45 sec.). Heat to 55–60°, and complete titrn by adding the $KMnO_4$ soln until faint pink persists 30 sec. Add last 0.5–1 ml dropwise, letting each drop decolorize before adding next. Det. excess of soln (usually 0.03–0.05 ml) required to impart pink color to same vol. of acid boiled and cooled to 55–60°. (In potentiometric titrns, correction is negligible if end point is approached slowly.)

(2) Transfer ca 0.3 g As_2O_3 (dried at 110°) to 400 ml beaker. Add 10 ml cool 20% NaOH soln and let stand until dissolved, stirring occasionally. Add 100 ml H_2O, 10 ml HCl (sp. gr. 1.18), and 1 drop $0.0025M$ KIO_3 or KI. Titr. with the $KMnO_4$ soln until faint pink persists 30 sec., adding last 1–1.5 ml dropwise and letting each drop decolorize before adding next. Det. by blank test with all reagents, except As_2O_3, vol. $KMnO_4$ soln (usually ca 0.03 ml) required to duplicate pink color of end point. (End point may also be taken with ferrous phenanthroline indicator (**c**), in which case 1 drop $0.025M$ soln of the indicator is added as end point approaches.) Det. blank correction. Titrn can also be conducted potentiometrically.

(**b**) *Ferric sulfate soln.*—Dissolve 135 g $FeNH_4(SO_4)_2.12H_2O$ or 55 g anhyd. $Fe_2(SO_4)_3$ in H_2O, and dil. to 1 L. Det. $Fe_2(SO_4)_3$ in stock supply by strong ignition to Fe_2O_3. Titr. 50 ml of the $Fe_2(SO_4)_3$ soln, acidified with 20 ml $4N$ H_2SO_4, with the $KMnO_4$ soln, and use this titer as zero-point correction.

(**c**) *Ferrous phenanthroline indicator.*—Dissolve 0.7425 g *o*-phenanthroline.H_2O in 25 ml $0.025M$ $FeSO_4$ soln (6.95 g $FeSO_4.7H_2O$/L).

29.044 DETERMINATION

Filter the Cu_2O in gooch, and wash beaker and ppt thoroly. Transfer asbestos pad to beaker with glass rod. Add 50 ml of the $Fe_2(SO_4)_3$ soln and stir vigorously until Cu_2O is completely dissolved. Examine for complete soln, holding beaker above eye level. Cu_2O must be quantitatively transferred; if necessary, immerse crucible in soln and make sure adhering Cu_2O is dissolved. Remove crucible with glass rod and wash with H_2O. Add 20 ml $4N$ H_2SO_4 and titr. with the std $KMnO_4$ soln. As end point approaches, add 1 drop of the ferrous phenanthroline indicator. At end point, brownish soln changes to green. Obtain wt reducing sugar equiv. to wt Cu from **43.012.**

29.045 *By Electrolytic Deposition from Nitric Acid Solution (26)*

Decant hot soln, **29.039,** thru asbestos mat in gooch, and wash beaker and ppt thoroly with hot H_2O. Transfer asbestos mat from crucible to beaker with glass rod and rinse crucible with 14 ml HNO_3 (1+1), letting rinsings flow into beaker. After Cu_2O dissolves, dil. to 100 ml, heat to boiling, and continue boiling ca 5 min. to remove oxides of N. Cool, filter, transfer to 250 ml beaker, and dil. to 200 ml. Add 1 drop $0.1N$ HCl and mix thoroly.

For electrolysis use cylindrical electrodes of Pt gauze, ca 1.5″ and 2″ diam., resp., and ca 1.75″ high, thoroly cleaned, ignited, cooled in desiccator, and weighed. Insert electrodes in Cu soln so that surface of cathode clears anode by at least 5 mm, and both electrodes almost touch bottom of beaker. Cover with split watch glass to avoid loss by spattering. Electrolyze with current of 0.2–0.4 ampere until deposition is complete, usually overnight. (Wash down sides of beaker and watch glass with H_2O, thus raising level of soln and exposing new surface of cathode; if new surface shows deposit of Cu, electrolysis is not complete.)

Without interrupting current, slowly lower beaker and at same time wash electrodes with stream of H_2O. Immediately immerse electrodes in another beaker of H_2O and break current. (Washing may also be accomplished by use of siphon; H_2O is added as soln is removed. Displacement of the HNO_3 soln is complete when current ceases to flow.) Rinse cathode with alcohol and dry few min. in oven at 100°. Cool in desiccator and weigh.

Electrolyte may be stirred by rotating anode or mechanical stirrer. In this case current may be increased to 1–2 amperes, thus shortening time

required for complete deposition of Cu to ca 1 hr.

If extreme care is taken to avoid spattering, the Cu_2O can be dissolved by letting the HNO_3 flow down walls of crucible. Keep crucible covered as much as possible with small watch glass. Collect filtrate in beaker, and wash watch glass and tip of pipet with jet of H_2O. Continue as above, beginning "dil. to 100 ml . . . " Obtain wt reducing sugars equiv. to wt Cu from **43.012.**

III. Ofner Volumetric Method (27)—Official

(For materials contg small quantities of invert sugar in presence of sucrose)

29.046 REAGENTS

(**a**) *Copper soln.*—Dissolve 5.0 g $CuSO_4.5H_2O$, 10.0 g anhyd. Na_2CO_3, 300 g pulverized Rochelle salt, and 50 g $Na_2HPO_4.12H_2O$ (or 19.8 g anhyd. salt) in ca 900 ml H_2O, warming finally if necessary. When completely dissolved, continue heating 2 hr on H_2O bath to destroy mold spores. Cool, and adjust vol. to 1 L. Treat with active C or kieselgühr and filter, or filter directly thru fine porosity fritted glass. Det. Cu content by electrolysis and adjust so that soln contains 63.4 mg Cu/50 ml. Store in dark place.

(**b**) *Sodium thiosulfate std soln.*—Dissolve 4.00 g crystals and dil. to 500 ml or, preferably, prep. stock soln contg, in 500 ml, 20.0 g crystals and 1 ml 1*N* NaOH or 0.1 g Na_2CO_3. Dil. 100 ml of this stock soln to 500 ml as required. Stdze by titrn against I soln, (**c**).

(**c**) *Iodine std soln.*—0.03230*N*. Dissolve 2.05 g pure I in ca 10 g iodate-free KI dissolved in few ml H_2O. Dil. to exactly 500 ml and store in dark place.

(**d**) *Starch soln.*—Rub 2.5 g sol. starch and ca 10 mg HgI_2 in little H_2O. Dissolve in ca 500 ml boiling H_2O.

29.047 DETERMINATION

Dissolve 20 g sample in H_2O and dil. to exactly 100 ml. Transfer 50 ml aliquot contg not >20 mg invert sugar to 300 ml erlenmeyer and add 50 ml of the Cu soln (**a**). Mix well, add 50–100 mg pumice or talcum powder, and bring to boil on asbestos gauze plate in 4–5 min. Judge initial time of boiling, not when bubbles arise from bottom of flask, but when they burst at surface in considerable number. Continue boiling exactly 5 min.

Cool without agitation by immersion in cold H_2O. Add 1 ml HOAc. Add, with continuous agitation, accurately measured vol. of the I soln, 5–30 ml according to quantity of Cu reduced, making sure that excess is finally present. Pour 15 ml 1*N* HCl down wall of flask from graduated cylinder. Stopper flask and let I react ca 2 min., agitating occasionally. Titr. excess I with the $Na_2S_2O_3$ soln, adding starch as end point approaches. Deduct vol. excess I from vol. added. Obtain quantity of invert sugar present by applying proper correction to vol. I soln consumed, **43.013.** After applying correction, 1 ml 0.0323*N* I consumed = 1 mg invert sugar.

29.048 *IV. Meissl-Hiller Gravimetric Method—Official*

(For materials contg >1.5% invert sugar and <98.5% sucrose)

Prep. soln of suitable concn of sample, clarify with neutral $Pb(OAc)_2$, and remove excess Pb. Prep. series of solns in large test tubes by adding 1, 2, 3, 4, and 5 ml of this soln to each tube successively. Add 5 ml mixed reagent, **29.035,** to each, heat to boiling, boil 2 min., and filter. Note vol. sugar soln that gives filtrate that is lightest in tint but still distinctly blue. Place 20 times this vol. of the sugar soln in 100 ml vol. flask, dil. to mark, and mix well. Place 50 ml of this dild soln and 50 ml reagent in 250 ml beaker, heat mixt. at such rate that ca 4 min. is required to bring it to b.p., and boil exactly 2 min. Add 100 ml cold, recently boiled H_2O. Filter immediately thru asbestos, **29.038,** and det. Cu by one of methods described in **29.040–29.045.** Calc. result by following formulas and table of factors of Meissl and Hiller, **43.014.**

Let Cu = wt Cu obtained; P = polarization of sample; W = wt sample in 50 ml soln used for detn; and F = factor obtained from table for conversion of Cu to invert sugar.

Then $Cu/2 = Z$, approx. wt invert sugar; $Z \times 100/W = Y$, approx. % invert sugar; $100P/(P+Y) = R$, approx. % sucrose in mixt. of sugars; $100 - R = I$, approx. % invert sugar; and CuF/W = % invert sugar.

Use factor F for calcg Cu to invert sugar, **43.014.** *Example:* Polarization of a sugar is 86.4, and 50 ml soln contg 3.256 g sample gives 0.290 g Cu.

$$Cu/2 = 0.290/2 = 0.145 = Z.$$

$$Z \times 100/W = 0.145 \times 100/3.256 = 4.45 = Y.$$

$$100P/(P+Y) = 8640/(86.4+4.45) = 95.1 = R$$

$$100 - R = 100 - 95.1 = 4.9 = I.$$

$$R:I = 95.1:4.9.$$

Table shows that vertical column headed 150 is nearest to Z, 145, and horizontal column headed 95.5 is nearest to ratio of R to I, 95.1:4.9. Where these columns meet find factor 51.2, which enters into final calcn:

$$CuF/W = 0.290 \times 51.2/3.256$$

$$= 4.56\% \text{ in vert sugar.}$$

V. Quisumbing-Thomas Method (28)—First Action

29.049 REAGENTS

(a) *Copper sulfate soln.*—Dissolve $CuSO_4.5H_2O$ in hot H_2O to make satd soln, and filter. Det. Cu electrolytically and dil. soln so that 25 ml contains 525 mg Cu (41.2 g $CuSO_4.5H_2O$/500 ml soln).

(b) *Alkaline tartrate soln.*—Dissolve NaOH in equal wt H_2O and let stand several days until insol. carbonates and other impurities settle out. Siphon off clear soln and establish its alky by titrn with std acid. Dissolve 173 g highest purity Rochelle salt in H_2O in 500 ml vol. flask and add calcd quantity of the NaOH soln so that 500 ml of this alk. tartrate soln contains exactly 65 g NaOH. Dil. to mark with H_2O.

29.050 DETERMINATION

Measure exactly 25 ml each of the $CuSO_4$ and alk. tartrate solns into 400 ml Pyrex beaker, diam. ca 9 cm. Add 50 ml sugar soln contg prefer, ably 50–150 mg sugar. Cover beaker with watch glass and place in H_2O bath held at 80°.

After digesting exactly 30 min., filter the Cu_2O by suction thru mat of asbestos in gooch. Wash ppt with H_2O; then with aid of stirring rod transfer asbestos mat and Cu_2O back into beaker in which reduction took place. Rinse inside of crucible and lip of beaker with 10 ml *soln of 240.9 g $FeNH_4(SO_4)_2.12H_2O$ and 200 ml H_2SO_4 dissolved in H_2O and dild to 1 L.* (Cool the dil. H_2SO_4 before adding the salt.) Receive rinsings in beaker contg the Cu_2O. Holding crucible over beaker, stir contents of beaker thoroly with stirring rod until Cu_2O dissolves. Wash crucible with ca 25 ml hot H_2O (80°), receiving washings in beaker. Stir contents of beaker and then raise beaker to see if any undissolved particles of Cu_2O are resting on bottom. If so, press out each one with point of stirring rod until all dissolve. Add ca 125 ml more hot H_2O. Add 1 drop of *soln of 0.15 g o-phenanthroline.H_2O and 0.07 g $FeSO_4$ in 10 ml H_2O.* Titr. at once, with continual stirring, with 0.05*N* $KMnO_4$. (In long titrn preferably add indicator just before end point is reached.) Stdze $KMnO_4$ as in **29.043.** Calc. wt sugar from tables, **43.019.**

VI. Berlin Institute Method (29)—First Action

29.051 REAGENT

Müller's soln.—Dissolve 35 g $CuSO_4.5H_2O$ in 400 ml boiling H_2O. Separately dissolve 173 g Rochelle salt and 68 g Na_2CO_3 in 500 ml boiling H_2O. Cool, mix the 2 solns, and dil. to 1 L. Shake with small amount of C and filter. If ppt forms on storage, refilter.

29.052 DETERMINATION

Select quantity of sample (10 g or less) contg not >30 mg invert sugar. Pipet 10 ml Müller's soln and 100 ml of the sugar soln into 300 ml flask and cover. Mix thoroly and heat exactly 10 min. in H_2O bath boiling so vigorously that immersion of flask does not interrupt boiling. Place flask so that H_2O level is at least 2 cm above surface of liquid in flask. After heating period, cool flask rapidly without agitation. Add 5 ml 5*N* HOAc to cooled soln, mix, and immediately add excess 0.0333*N* I soln (20–40 ml) from buret. After all Cu_2O ppt dissolves, titr. excess I with 0.0333*N* $Na_2S_2O_3$.

Apply following corrections to ml I soln consumed: (1) ml I required in blank with H_2O instead of sugar soln; (2) ml I required by sugar soln in detn conducted without heating; (3) 2.0 ml I for reducing action of 10 g sucrose or proportionate correction for smaller amount sucrose. After these corrections 1 ml 0.0333*N* I = 1 mg invert sugar.

Dextrose—Chemical Methods

29.053 *Lane-Eynon General Volumetric Method—Official*

Proceed as in **29.037,** referring titer to **43.017** or **43.018.**

29.054 *Munson-Walker General Method—Official*

Proceed as in **29.039** and obtain wt dextrose from **43.011** or **43.012.**

Shaffer-Somogyi Micro Method—Official

29.055 REAGENTS

(a) *Shaffer-Somogyi carbonate 50 reagent, 5 g KI.*—Dissolve 25 g each of anhyd. Na_2CO_3 and Rochelle salt in ca 500 ml H_2O in 2 L beaker. Add thru funnel with tip under surface, with stirring, 75 ml of soln of 100 g $CuSO_4.5H_2O$/L. Add 20 g $NaHCO_3$, dissolve, and add 5 g KI. Transfer soln to 1 L vol. flask, add 250 ml 0.100*N* KIO_3 (3.567 g dissolved and dild to 1 L), dil. to vol., and filter thru fritted glass. Age overnight before use.

(b) *Iodide-oxalate soln.*—Dissolve 2.5 g KI and 2.5 g $K_2C_2O_4$ in H_2O and dil. to 100 ml. Prep. fresh weekly.

(c) *Thiosulfate std soln.*—0.005*N*. Prep. daily from stdzd stock 0.1*N* soln, **42.035–42.036.**

29.056 DETERMINATION

Pipet 5 ml soln contg 0.5–2.5 mg dextrose into 25×200 mm test tube. Add 5 ml reagent and mix well by swirling. Prep. blank, using 5 ml H_2O and 5 ml reagent. Place tubes, capped with bulb or funnel, in boiling H_2O bath 15 min. Remove tubes carefully without agitation to running H_2O cooling bath 4 min. Remove caps and add down side of each tube 2 ml KI-$K_2C_2O_4$ soln and then 3 ml 2*N* H_2SO_4 (56 ml/L). (Do not agitate solns while

alk.) Mix thoroly to insure that all Cu_2O is dissolved, and let stand in cold H_2O bath 5 min., mixing twice during that time. Titr. with 0.005N $Na_2S_2O_3$, using starch indicator, **29.046(d)**. Subtract titrn of test soln from that of blank and det. amount dextrose in 5 ml soln from **29.057**.

Make control detns with known amounts of dextrose and apply corrections for any deviations from tabulated equivs.

29.057 *Shaffer-Somogyi Dextrose-Thiosulfate Equivalents**

mg dextrose = (0.1099) (ml 0.005N $Na_2S_2O_3$) +.048

ML 0.005N $Na_2S_2O_3$	TENTHS ML 0.005N THIOSULFATE									
	0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
	MG DEXTROSE IN 5 ML OF SOLN									
3	.378	.389	.400	.411	.422	.432	.444	.455	.466	.477
4	.488	.499	.510	.521	.532	.543	.554	.565	.576	.587
5	.598	.608	.619	.630	.641	.652	.663	.674	.685	.696
6	.707	.718	.729	.740	.751	.762	.773	.784	.795	.806
7	.817	.828	.839	.850	.861	.872	.883	.894	.905	.916
8	.927	.938	.949	.960	.971	.982	.993	1.004	1.015	1.026
9	1.037	1.048	1.059	1.070	1.081	1.092	1.103	1.114	1.125	1.136
10	1.147	1.158	1.169	1.180	1.191	1.202	1.213	1.224	1.235	1.246
11	1.257	1.268	1.279	1.290	1.301	1.312	1.323	1.334	1.345	1.356
12	1.367	1.378	1.389	1.400	1.411	1.422	1.433	1.444	1.455	1.466
13	1.477	1.488	1.499	1.510	1.521	1.532	1.543	1.554	1.565	1.576
14	1.587	1.598	1.609	1.620	1.631	1.642	1.653	1.664	1.675	1.686
15	1.697	1.707	1.718	1.729	1.740	1.751	1.762	1.773	1.784	1.795
16	1.806	1.817	1.828	1.839	1.850	1.861	1.872	1.883	1.894	1.905
17	1.916	1.927	1.938	1.949	1.960	1.971	1.982	1.993	2.004	2.015
18	2.026	2.037	2.048	2.059	2.070	2.081	2.092	2.103	2.114	2.125
19	2.136	2.147	2.158	2.169	2.180	2.191	2.202	2.213	2.224	2.235
20	2.246	2.257	2.268	2.279	2.290	2.301	2.312	2.323	2.334	2.345
21	2.356	2.367	2.378	2.389	2.400	2.411	2.422	2.433	2.444	2.455
22	2.466	2.477	2.488	2.499	2.510	2.521	2.532	2.543	2.554	2.565

* J. Biol. Chem. **100**, 695 (1933); **160**, 61 (1945); J. Assoc. Offic. Agr. Chemists **42**, 341 (1959); **43**, 645 (1960).

Folin and Wu Micro Method (30)—First Action

29.058 REAGENTS

(**a**) *Phosphomolybdic acid soln.*—Add 200 ml 10% NaOH soln and 200 ml H_2O to 35 g molybdic acid and 5 g Na tungstate in 1 L beaker. Boil vigorously 20–40 min. Cool, dil. to ca 350 ml, add 125 ml H_3PO_4 (85%), dil. to 500 ml, and mix.

(**b**) *Alkaline copper soln.*—Dissolve 40 g Na_2CO_3 in ca 400 ml H_2O and transfer to 1 L vol. flask. Dissolve 7.5 g tartaric acid in this soln and then 4.5 g $CuSO_4.5H_2O$; mix, and dil. to vol. If sediment forms on standing, decant and use clear supernatant.

(**c**) *Dextrose std soln.*—(1) *Stock soln.*—Dissolve 1.0 g highest purity anhyd. dextrose in ca 50 ml filtered 0.25% benzoic acid soln and dil. to vol. in 100 ml vol. flask with the benzoic acid soln. This std keeps indefinitely.

(2) *Dilute std soln.*—Transfer 2.0 ml of the stock soln (1) to 100 ml vol. flask, dil. to vol. with H_2O (or 0.25% benzoic acid soln if soln is to be kept for any length of time), and mix well.

29.059 DETERMINATION

Transfer 2 ml sample soln to Folin-Wu blood sugar test tube. Add 2 ml alk. Cu soln. Surface of mixt. must reach constricted part of tube. Transfer tube to boiling H_2O bath and heat 6 min. Transfer to cold H_2O bath and cool without shaking 2–3 min. Add 2 ml of the phosphomolybdic acid soln. When Cu_2O dissolves, dil. soln to 25 ml mark with the phosphomolybdic acid soln dild 1+4, insert rubber stopper, and mix. Let stand 10–15 min., transfer to colorimeter tube, and read within 15 min. in photoelec. colorimeter at 420 mμ.

Make parallel detns on 2 ml H_2O as blank and on 2 ml of the dil. std dextrose soln. Express reading as function of absorbance (−log transmittance) and calc. unknown concn, $c = c'A/A'$, where c' is concn of std, A is absorbance of unknown, and A' is absorbance of std.

Levulose—Chemical Methods—Official

29.060 *Lane-Eynon General Volumetric Method*

Proceed as in **29.037**, referring titer to **43.017** or **43.018**.

29.061 *Munson-Walker General Method*

Proceed as in **29.039** and **29.045**, and obtain wt levulose equiv. to wt Cu from **43.012**.

Jackson-Mathews Modification of Nyns Selective Method (31)

29.062 REAGENT

Ost soln.—Dissolve 250 g anhyd. K_2CO_3 in ca 700 ml hot H_2O, add 100 g pulverized $KHCO_3$, and agitate mixt. until completely dissolved. Cool, and add, with very vigorous agitation, soln of 25.3 g $CuSO_4.5H_2O$ in 100–150 ml H_2O. Dil. to 1 L and filter.

29.063 DETERMINATION

Transfer 50 ml of the Ost soln to 125 ml erlenmeyer and pipet in vol. sample soln contg not >92 mg levulose or its equiv. of levulose-dextrose mixt. (dextrose has ca $\frac{1}{12}$ reducing power of levulose). Add H_2O to 70 ml. Immerse in H_2O bath, regulated at 55° preferably within 0.1°. Digest exactly 75 min., swirling at 10 or 15 min. intervals.

Filter pptd Cu_2O on closely packed asbestos-mat gooch, and wash flask and ppt thoroly without attempting to transfer ppt quantitatively. Det. Cu by one of methods described in **29.041–29.045.** As it is usually difficult to transfer Cu ppt quantitatively from erlenmeyer, select method of Cu analysis in which total Cu is dissolved in HNO_3 and detd by electrolysis or $Na_2S_2O_3$ titrn, or in $Fe_2(SO_4)_3$ soln followed by $KMnO_4$ titrn as in **29.044.**

See **43.016** for levulose equiv. If sample contained dextrose in addn to levulose, analytical result is not true but "apparent" levulose, as dextrose has appreciable reducing action under conditions of analysis. To det. correction for dextrose, analyze sample also for total reducing sugars and compute true dextrose and levulose by series of approximations. Calc. % reducing sugars in original sample and similarly % "apparent" levulose. Difference between these 2 percentages is "apparent" dextrose. Divide apparent dextrose by factor 12.4 and deduct result from apparent levulose to obtain new approximation to true levulose. Deduct new levulose % from total reducing sugar % to obtain more nearly correct value for true dextrose and again divide by 12.4. Deduct quotient from original value of "apparent" levulose and continue approximation in same manner until % levulose remains essentially unaltered by 2 successive approximations.

If original sample contd sucrose, det. by means of Clerget procedure, **29.026.** Correct Cu for reducing action of sucrose before referring to the table, **43.016.** 1, 2, 3, 4, and 5 g sucrose under conditions of the analysis ppt 3.3, 5.7, 7.4, 8.5, and 9.0 mg Cu, resp.

Maltose—Chemical Methods—Official

29.064 *Lane-Eynon General Volumetric Method*

Proceed as in **29.037**, referring titer to **43.017** or **43.018.**

29.065 *Munson-Walker General Method*

Proceed as in **29.039** and **29.040**, and obtain wt maltose equiv. to wt Cu_2O from **43.011.**

Lactose—Chemical Methods—Official

29.066 *Lane-Eynon General Volumetric Method*

Proceed as in **29.037**, referring titer to **43.017** or **43.018.**

29.067 *Munson-Walker General Method*

Proceed as in **29.039** and **29.040**, and obtain wt lactose equiv. to wt of Cu_2O from **43.011.**

29.068 Arabinose, Galactose, and Xylose and Other Sugars—First Action

Proceed as in **29.056**, using appropriate heating time and equation for calcn from **29.069.** Make control detns with known amounts of the sugar and apply corrections for any deviations from equations.

29.069 *Shaffer-Somogyi Sugar-Thiosulfate Equivalents*

y = mg sugar in 5 ml; x = ml $0.005N$ $Na_2S_2O_3$.

SUGAR	HEATING TIME MIN.	EQUATION
L-Arabinose	30	$y = 0.1234x + 0.060$
Dextrose	15	$y = 0.1099x + 0.048$
D-Galactose	30	$y = 0.1332x + 0.033$
Lactose	35	$y = 0.2031x + 0.030$
Levulose	15	$y = 0.1113x + 0.079$
Maltose	30	$y = 0.2199x + 0.0725$
D-Mannose	35	$y = 0.1148x + 0.084$
D-Ribose	25	$y = 0.1381x + 0.098$
L-Sorbose	15	$y = 0.1244x + 0.116$
D-Xylose	30	$y = 0.1103x + 0.044$

Unfermentable Reducing Substances (32)—Official

(Applicable to molasses)

29.070 REAGENTS

(**a**) *Baker's yeast, free from starch.*—Fleischmann, sold in 1 lb units by Standard Brands,

Inc., is suitable. It keeps fresh for few days if kept in refrigerator.

(**b**) *Neutral lead acetate soln.*—Dissolve 20 g $Pb(OAc)_2.3H_2O$ in H_2O and dil. to 100 ml.

(**c**) *Soxhlet soln.*—*See* **29.035**(**a**) and (**b**).

(**d**) *Potassium iodide soln.*—Dissolve 20 g KI in H_2O and dil. to 100 ml.

(**e**) *Thiosulfate std soln.*—0.1*N*. *See* **42.035** and **42.036.**

29.071 FERMENTATION

Transfer 12 g blackstrap molasses (or 8 g high-test molasses) to 500 ml vol. flask, using 75 ml H_2O in all. Add 25 g of the fresh yeast, coarsely chopped, and mix thoroly with molasses soln. Close flask with stopper provided with delivery tube, other end of which dips ca 1 cm below surface of H_2O in beaker; or use any other type of fermentation trap. Place flask in H_2O bath kept at 30° and let ferment at least 4 hr, shaking occasionally. (Incubator may be used and flask left overnight.)

When fermentation is complete, dil. mixt. with H_2O, clarify with 15 ml of the neutral $Pb(OAc)_2$ soln, dil. to mark at 20°, add teaspoonful of Filter-Cel, shake well, and filter, discarding first few ml. Delead entire filtrate with ca 0.5 g anhyd. $K_2C_2O_4$ and filter again with aid of Filter-Cel. Test filtrate for Pb. If necessary add more $K_2C_2O_4$ and refilter.

29.072 DETERMINATION

Transfer 25 ml final filtrate to 250 ml erlenmeyer, mix with 20 ml of the combined Soxhlet soln, and wash wall of flask down with 5 ml H_2O, making 50 ml in all. Add few pieces of ignited pumice stone and place flask on wire gauze covered with asbestos plate having center hole slightly smaller than bottom of flask. Heat with Bunsen burner or, preferably, elec. heater with temp. control. Heat to boiling in 3 min. and boil gently exactly 2 min. longer. Close flask immediately with stopper provided with Bunsen valve and cool quickly under H_2O tap to prevent reoxidation. Add 15 ml of the KI soln and then 10 ml H_2SO_4 (1+3). Titr. liberated I at once with 0.1*N* $Na_2S_2O_3$, adding starch indicator toward end of titrn.

Det. blank with 75 ml H_2O instead of molasses soln, adding yeast, etc., as above. Deduct titer of sample from titer of blank, and find mg invert sugar corresponding to difference from table, **29.073.** Result, divided by 6 (in case of high-test molasses, by 4) gives directly % unfermentable reducing substances in the molasses, in terms of invert sugar.

29.073 *Milligrams Invert Sugar Corresponding to 0.1N Thiosulfate*

0.1*N* THIOSULFATE	INVERT SUGAR	0.1*N* THIOSULFATE	INVERT SUGAR
ml	*mg*	*ml*	*mg*
1	3.2	14	47.3
2	6.4	15	50.8
3	9.7	16	54.3
4	13.0	17	58.0
5	16.4	18	61.8
6	19.8	19	65.5
7	23.2	20	69.4
8	26.5	21	73.3
9	29.9	22	77.2
10	33.4	23	81.2
11	36.8	24	85.2
12	40.3	25	89.2
13	43.8	—	—

CONFECTIONERY

29.074 Preparation of Sample—Official

If composition of entire sample is desired, grind and mix thoroly. If sample is composed of layers or of distinctly different portions and it is desired to examine these individually, sep. with knife or other mechanical means as completely as possible, and grind and mix each portion thoroly.

29.075 Moisture—Official—*See* **29.005, 29.006, 29.007,** or **29.008**

29.076 Ash—Official—*See* **29.012** or **29.013**

29.077 Soluble and Insoluble Ash—Official—*See* **29.015**

29.078 Alkalinity of Soluble Ash—Official—*See* **29.016**

29.079 Alkalinity of Insoluble Ash—Official—*See* **29.017**

29.080 Mineral Adulterants in Ash—First Action—*See* **29.018**

29.081 Nitrogen—Official

Det. N in 2–5 g sample as in **2.036,** using larger quantity of H_2SO_4 if necessary for complete digestion.

Sucrose—Polarimetric Methods

29.082 *In Absence of Raffinose—Official—See* **29.025, 29.026,** or **29.031**

Sucrose—Chemical Methods

29.083 *By Reducing Sugars Before and After Inversion—Official—See* **29.032**

29.084 Commercial Glucose—Procedure —*See* 29.033 or 29.034

29.085 Starch—First Action

Measure 25 ml of soln of uniform mixt. (representing 5 g sample) into 300 ml beaker, or add to beaker 5 g finely ground sample (previously extd with ether if sample contains much fat); add enough H_2O to make 100 ml; heat to ca 60° (avoiding, if possible, gelatinizing starch); and let stand ca 1 hr, stirring frequently to secure complete soln of sugars. Transfer to wide-mouth bottle, rinse beaker with little warm H_2O, and cool. Add equal vol. alcohol, mix, and let stand at least 1 hr.

Centrifuge until ppt is closely packed on bottom of bottle and decant supernatant thru hardened filter. Wash ppt with successive 50 ml portions of alcohol, 50% by vol., by centrifuging and decanting thru filter until washings are sugar-free by following test: Add to test tube few drops of the washings, 3 or 4 drops *20% alc. α-naphthol soln*, and 2 ml H_2O. Shake well, tip tube, let 2–5 ml H_2SO_4 flow down side of tube, and then hold tube upright. If sugar is present, interface of 2 liquids is colored faint to deep violet; on shaking, whole soln becomes blue violet. Transfer residue from bottle and hardened filter to large flask and proceed as in **22.045**.

Ether Extract—First Action

29.086 *Continuous Extraction Method*

Measure 25 ml 20% mixt. or soln into very thin, readily frangible glass evapg shell, or thin Pb or Sn foil dish contg 5–7 g freshly ignited asbestos fiber; or, if possible to obtain uniform sample, weigh 5 g mixed finely divided sample into dish and wash with H_2O onto the asbestos in evapg shell, using, if necessary, small portion of the asbestos fiber on stirring rod to transfer last traces of sample from dish to shell.

Dry to constant wt at 100°, cool, wrap glass dish loosely in smooth paper, crush into rather small fragments between fingers, and carefully transfer crushed mass, including paper, to extn tube or fat extn cartridge. If metal dish is used, cut into small pieces and place in extn tube. Ext. with anhyd. ether or petr. ether (b. p. 45–60° and without weighable residue) in continuous extn app. at least 25 hr. In most cases it is advisable to remove substance from extractor after first 12 hr, grind with sand to fine powder, and re-ext. remaining 13 hr. Transfer ext. to weighed flask, evap. solvent, and dry to constant wt at 100°.

29.087 *Roese-Gottlieb Method*

Introduce 4 g sample, or quantity of uniform soln equiv. to this wt dry substance, into Mojonnier fat extn tube or similar app.; dil. to 10 ml with H_2O, add 1.25 ml NH_4OH, and mix thoroly. Add 10 ml alcohol and mix; then add 25 ml ether and shake vigorously ca 30 sec.; and finally add 25 ml petr. ether (b. p. <60°) and shake again ca 30 sec. Let stand 20 min. or until sepn of liquids is complete.

Draw off as much as possible of ether-fat soln (usually 0.5–0.8 ml is left) into weighed flask thru small, rapid filter. (Weigh flask with similar one as counterpoise.) Again ext. liquid remaining in tube, this time with 15 ml each of ether and petr. ether; shake vigorously ca 30 sec. with each solvent and let settle. Proceed as above, washing mouth of tube and filter with few ml of mixt. of equal parts of the 2 solvents (previously mixed and freed from deposited H_2O).

For greater degree of accuracy, repeat extn. If previous solvent-fat solns have been drawn off closely, third extn usually yields not >1 mg fat, or ca 0.02% on 4 g charge. Evap. solvent slowly on steam bath and then dry fat in boiling H_2O oven to constant wt. Test purity of fat by dissolving in little petr. ether. If residue remains, wash fat out completely with petr. ether, dry residue, weigh, and deduct wt.

29.088 Paraffin—First Action

Add to solvent ext. in flask, **29.086 or 29.087,** 10 ml alcohol and 2 ml NaOH soln (1+1); connect flask with reflux condenser; and heat 1 hr on H_2O bath, or until saponification is complete. Remove condenser and keep flask on bath until alcohol evaps and residue is dry. Dissolve residue as completely as possible in ca 40 ml H_2O and heat on bath, shaking frequently. Wash into separator, cool, and ext. with 4 successive portions of petr. ether, collecting exts in weighed flask or capsule. Evap. petr. ether and dry to constant wt at 100°. Any phytosterol or cholesterol present in fat would be extd with the paraffin but quantity is so insignificant that it may generally be disregarded.

29.089 Shellac (*33*)—Official

Place 50 g candy in 400 ml beaker. Add 50 ml mixt. of benzene and absolute alcohol (1+1), and cover with watch glass. Place beaker on steam bath, heat to boiling, and simmer few min., stirring occasionally. Decant liquid into tared, round 100 ml glass dish with flat bottom ca $2\frac{3}{4}''$ diam. Ext. once more with the benzene-alcohol mixt., and finally rinse with two 25 ml portions absolute alcohol, simmering and stirring each time. With moist sugar candy, avoid overheating to prevent pieces from sticking together.

Add each ext. to glass dish previously placed on steam bath. Evap. until alcohol is just removed, rotating dish as it goes to dryness in order to spread ext. uniformly over bottom surface. Avoid

baking the shellac on dish. If fat appears to be present, wash with three 15 ml portions petr. ether, stirring and warming. Decant thru rapid filter.

Add mixt. of 25 ml *isoamyl alcohol* (b.p. 129–132°) and 25 ml benzene to filter, and filter back into dish. Heat on steam bath with stirring, cool somewhat, and transfer soln with suspended matter to 125 ml separator. Rinse dish with 25 ml hot (ca 60°) H_2O, and add to separator; shake well, and filter wash H_2O if necessary. Repeat washing with H_2O *twice* (or until washings are colorless), rinsing dish well around sides with first portions of liquid. Finally, filter soln of the shellac into tared dish, rinsing separator and filters with little absolute alcohol. Evap. to dryness on steam bath, rotating dish to give uniform film.

If much fat was extd in original benzene extn, wash final shellac residue with 25 ml petr. ether, warming and stirring. Decant, dry on steam bath and in 100° oven, and weigh. After weighing, check for complete removal of sugars by thoroly rinsing dish and surface of shellac with hot H_2O, warming on steam bath, decanting, rinsing down with alcohol, and evapg with care to give uniform film on dish. Dry and reweigh.

29.090 Alcohol in Sirups Used in Confectionery ("Brandy Drops")—Official

Collect in beaker sirup from enough pieces of sample to yield 30–50 g, strain into weighed beaker, and weigh. Place sirup in 250–300 ml distg flask, dil. with half its vol. of H_2O, attach flask to vertical condenser, and distill almost 50 ml, or as much of liquid as possible without causing charring. Foaming may be prevented by adding little *tannin*, or piece of *paraffin* ca size of pea. Cool distillate, dil. to vol. with H_2O, and mix well. Det. sp. gr. as in **9.011**. Calc. % alcohol by wt in candy filling, using tables **43.021** and **43.023**.

HONEY

29.091 Preparation of Sample—Official

(a) *Liquid or strained honey.*—If sample is free from granulation, mix thoroly by stirring or shaking before weighing portions for detns; if granulated, place container, with stopper tight, in H_2O bath without submerging, and heat 30 min. at 60°; then if necessary heat at 65° until liquefied. Occasional shaking is essential. Mix thoroly, cool rapidly as soon as sample liquefies, and weigh portions for detns. Do not heat honey intended for diastatic detn. If foreign matter, such as wax, sticks, bees, particles of comb, etc., is present, heat sample to 40° in H_2O bath and strain thru cheesecloth in hot H_2O funnel before weighing portions for analysis.

(b) *Comb honey.*—Cut across top of comb, if sealed, and sep. completely from comb by straining thru No. 40 sieve. When portions of comb or wax pass thru sieve, heat sample as in (a) and strain thru cheesecloth. If honey is granulated in comb, heat until wax is liquefied; stir, cool, and remove wax.

Color Classification (*34*)—First Action

29.092 APPARATUS

(a) *Containers.*—French square bottles, Hazel Atlas catalog No. 2653, screw finish, clear glass, 1.5×1.5″, $2\frac{7}{32}$ oz.

(b) *Comparator.*—All-metal boxes, approx. 8×2×3″, divided by thin partitions into 5 square compartments, each of which has 2 windows, front and back, ca 1.2″ square. The 3 lighter glass stds (water white, extra white, and white) are mounted in 1 of comparator boxes on shelf against front windows in compartments 1, 3, and 5. The 3 darker stds (extra light amber, light amber, and amber) are mounted similarly in second comparator box. Place containers, (a), filled with H_2O (blanks) behind glass stds. With turbid honeys substitute containers filled with suspensions of diatomaceous earth (Hyflo Super-Cel) designated as "Cloudy 1," "Cloudy 2," and "Cloudy 3," contg 100, 200, and 400 mg/L H_2O, resp.

(c) *Glass stds.*—Use selected colored glasses tested and stdzd by U. S. Department of Agriculture to correspond with color stds for honey.

Complete grading set is available from Phoenix Precision Instrument Co., 3803 North 5th St., Philadelphia 40, Pa.

29.093 DETERMINATION

Place clear blanks or cloudy suspensions in back of glass stds in compartments 1, 3, and 5 of 1 or both comparators. Pour sample (must be free from granulation) into clean, dry container, **29.092**(a). Place sample container in compartment 2 or 4 of either comparator. Hold comparator at convenient distance from eye and view by diffused light (e.g., north or overcast sky, diffused light from W lamp, or white or daylight fluorescent lamp). Move sample from compartment to compartment, interchanging blanks with cloudy suspensions if necessary. Det. classification as follows: If sample is equal to water white std in hue, or not as red (i. e., yellower), classify as water white; if perceptibly redder than water white std in hue but not redder than extra white std, classify as extra white, etc. If redder in hue than amber std, classify as dark amber. Hue (amber quality or redness) is attribute of color in classification.

Moisture—Official

29.094 *Direct Drying*

Proceed as in **29.007** or **29.008**, using weighed quantity of sample sufficient to yield ca 1 g solids. Add, if necessary, few ml H_2O to incorporate sample thoroly with the sand. Dry at <70° (preferably 60°) under pressure not >50 mm Hg.

29.095 *By Means of Refractometer*

Det. refractometer reading of soln at 20° and obtain corresponding percentages of moisture from **29.096**. If detn is made at temp. other than 20°, correct reading to std temp. of 20° according to footnote.

H_2O at 20°, filter, and polarize immediately in 200 mm tube.

(**b**) *Constant direct polarization.*—Complete mutarotation as in **29.023**. If necessary to conserve sample, soln from tube used in immediate direct polarization (**a**) may be returned to flask. Make final reading at 20° in 200 mm tube.

(**c**) *Mutarotation.*—Difference between (**a**) and (**b**) is measure of mutarotation.

(**d**) *Direct polarization at 87°.*—Polarize soln obtained in (**b**) at 87° in jacketed 200 mm metal tube.

29.101 Invert Polarization—First Action

(**a**) *At 20°.*—Invert 50 ml soln, **29.100**, as in **29.025**(**b**) or (**c**) or **29.026**(**b**) or (**c**), and polarize at 20° in 200 mm tube.

(**b**) *At 87°.*—Polarize soln (**a**) at 87° in jacketed 200 mm metal tube.

29.096 *Chataway table showing relationship between refractive index, moisture content, and weight per gallon of honey*

REFRACTIVE INDEX AT 20°	MOISTURE	WT HONEY IN LBS/GALLON AT 20°		REFRACTIVE INDEX AT 20°	MOISTURE	WT HONEY IN LBS/GALLON AT 20°	
	per cent	*lb*	*oz*		*per cent*	*lb*	*oz*
1.5041	13.0			1.4935	17.2		
35	.2	12	1	30	.4	11	13
30	.4			25	.6		
25	.6			20	.8		
20	.8	12	½	15	18.0	11	12½
15	14.0			10	.2		
10	.2	12	0	05	.4		
05	.4			00	.6	11	12
00	.6			1.4895	.8		
1.4995	.8	11	15½	90	19.0	11	11½
90	15.0			85	.2		
85	.2			80	.4		
80	.4	11	15	76	.6	11	11
75	.6			71	.8		
70	.8	11	14½	66	20.0		
65	16.0			62	.2	11	10½
60	.2			58	.4		
55	.4	11	14	53	.6	11	10
50	.6			49	.8		
45	.8			1.4844	21.0		
40	17.0	11	13½				

Temp. corrections: Refractive Index, 0.00023/°C or 0.00013/°F; lbs/gallon, ½ oz/6°C or 11°F. If reading is made at temp. above 20° C (68° F) add the correction; if made below, subtract the correction. *Can. J. Research* 6, 532(1932); 8, 435(1933); *Canadian Bee J.* August 1935, p. 215; *J. Assoc. Offic. Agr. Chemists* 25, 99, 681(1942).

29.097 **Ash—Official**

Weigh 5–10 g honey into Pt dish, add few drops pure olive oil to prevent spattering, heat carefully until swelling ceases, and ignite at temp. not above dull redness (ca 600°) until white ash is obtained.

29.098 Soluble Ash—Official—*See* 29.015

29.099 Alkalinity of Soluble Ash—Official—*See* 29.016

29.100 Direct Polarization—First Action

(**a**) *Immediate direct polarization.*—Transfer 26 g honey to 100 ml vol. flask with H_2O, add 5 ml alumina cream, **29.021**(**b**), dil. to mark with

29.102 **Reducing Sugars—Official**

Dil. 10 ml soln, **29.100**, to 250 ml and det. reducing sugars in 25 ml of this soln by **29.037** or **29.039**. Calc. result to % invert sugar.

29.103 **Sucrose—Official**

(**a**) Calc. from data given in **29.100**(**b**) and **29.101**(**a**) if inversion is conducted as in **29.025**(**b**) or (**c**). Use formula given in **29.025**(**b**).

(**b**) Proceed as in **29.032**. To det. reducing

sugars after inversion, dil. 10 ml soln, **29.101,** with small quantity of H_2O, neutralize with Na_2CO_3, and dil. to 250 ml with H_2O. Use 50 ml of this soln, making detn as in **29.102.**

29.104 Levulose—First Action

Multiply direct reading at 87°, **29.100(d)**, by 1.0315 and from product subtract constant direct polarization at 20°, **29.100(b)**; divide difference by 2.3919 to obtain g levulose in normal wt honey. From this figure calc. % levulose in original sample, or det. levulose selectively by **29.063.**

29.105 Dextrose—First Action

To obtain approx. % dextrose, subtract % levulose, **29.104,** from % invert sugar, **29.102.**

To det. dextrose more closely, multiply % levulose, **29.104,** by factor 0.915, which gives its dextrose equiv. in Cu reducing power. Subtract figure obtained from that of reducing sugars, **29.102,** calcd as dextrose, to obtain % dextrose in sample. Because of difference in reducing powers of different sugars, sum of dextrose thus found and levulose, **29.104,** will be greater than quantity of invert sugar obtained in **29.102.**

29.106 Dextrin (Approximate)—Procedure

Using not >4 ml H_2O, transfer 8 g sample (4 g for dark-colored honeydew honey) to 100 ml vol. flask by letting sample drain from weighing dish into flask and then dissolving residue in 2 ml H_2O. Add this soln to flask, and rinse weighing dish with two 1 ml portions H_2O, adding few ml absolute alcohol each time before decanting. Fill flask to mark with absolute alcohol, shaking constantly. Set flask aside until dextrin collects on sides and bottom and liquid is clear.

Decant clear liquid thru filter paper and wash residue in flask with 10 ml alcohol, pouring washings thru same filter. Dissolve dextrin in flask with boiling H_2O and filter thru paper already used, receiving filtrate in weighed dish prepd as in **29.008.** Rinse flask and wash filter number of times with small portions of hot H_2O, evap. on H_2O bath, and dry to constant wt at 70° under pressure not >50 mm Hg.

After detg wt alcohol ppt, dissolve latter in H_2O and dil. to definite vol., using 50 ml H_2O for each 0.5 g ppt or part thereof.

Det. reducing sugars in soln both before and after inversion as in **29.032,** expressing results as invert sugar. Calc. sucrose from results thus obtained and subtract sum of reducing sugars before inversion and sucrose from wt total alcohol ppt to obtain wt dextrin.

Sugars—First Action

Chromatographic Method (35)

29.107 PRINCIPLE

By adsorption of honey sample on charcoal column, followed by elution into monosaccharide, disaccharide, and higher sugar fractions, interference of disaccharides in dextrose and levulose detns is eliminated. Elution is by progressively higher alcohol concns, followed by detn of individual monosaccharides, sucrose, and reducing disaccharides collectively as maltose, and trisaccharides and higher sugars collectively after hydrolysis.

29.108 PREPARATION AND STANDARDIZATION OF ADSORPTION COLUMN

Column is 22 mm o.d.×370 mm long, with 1 L spherical section and 35/20 ₸ spherical joint at top. Adsorbent is 1+1 mixt. of Darco G-60 charcoal and rapid filter-aid (Celite 545 or Dicalite 4200). Insert glass wool plug, wet from below, and add enough dry adsorbent to the dry tube (23–26 cm) to compress to 17 cm when vac. is applied with *gentle* tapping of column. Remove excess charcoal from walls of column, and add filter-aid layer at top with *gentle* packing (1–1.5 cm). Wash column with 500 ml H_2O and 250 ml 50% alcohol, and let stand overnight with 50% alcohol on it. Flow rate should be 5.5–8.0 ml/min. with H_2O at 9 lb/sq.in. pressure. Slower flow rates delay analyses excessively.

Alcohol content of eluting solns must be adjusted to retentive power of charcoal used. Wash column alcohol-free with 250 ml H_2O, quantitatively add 10 ml soln of 1.000 g anhyd. glucose to top, and draw it into column with suction (do not let dry). Add 300 ml H_2O to top, break suction, apply pressure (10 lb/sq.in. max.), and collect eluate in five 50 ml portions in tared beakers. Include 10 ml from sample introduction in first 50 ml fraction. Evap. fractions on steam bath, dry in vac. oven at 80–100°, and weigh.

Decant remaining H_2O from top of column, pass 50 ml 50% alcohol and then 250 ml H_2O thru column, and repeat chromatography, using 1.000 g anhyd. glucose in 10 ml 1% alcohol, washing with 250 ml 1% alcohol as above. Select as solvent *A* that which removes glucose in 150 ml. Repeat chromatography with 2% alcohol if necessary.

Wash column with 250 ml H_2O and then 20 ml 5% alcohol. To top, add 10 ml 5% alcohol soln contg 100 mg maltose and 100 mg sucrose. Elute as above with 250 ml 5% alcohol, weighing evapd 50 ml portions of filtrate. Repeat, if necessary, with 7%, 8%, and 9% alcohol to find solvent *B* that will elute at least 98% disaccharides in 200 ml. Solvent *A* previously selected must not elute disaccharides. Combinations found satisfactory

with various charcoals are 1%, 7%; 2%, 8%; 2%, 9%. At conclusion, pass 100 ml 50% alcohol thru column, and store under layer of this solvent.

29.109 PREPARATION OF FRACTIONS

Wash column with 250 ml H_2O and decant any supernatant. Pass 20 ml solvent *A* thru column, and discard. Dissolve 1 g sample in 10 ml solvent *A* in 50 ml beaker. Transfer sample (using long-stem funnel) onto column, and force into column. Use 15 ml solvent *A* to rinse beaker and funnel, and add to column. Collect all eluate, beginning with sample introduction, in 250 ml vol. flask. Add 250 ml solvent *A*, and collect exactly 250 ml total (fraction *A*, monosaccharides). Decant excess solvent from top, add 265–270 ml solvent *B*, and collect 250 ml in vol. flask (fraction *B*, disaccharides). Decant excess, add 110 ml 50% alcohol (solvent *C*), and collect 100 ml in vol. flask (fraction *C*, higher sugars). Mix each fraction thoroly. Column may be stored indefinitely, outlet closed, under 50% alcohol. Discard after 8 uses.

Fructose

29.110 REAGENTS

Use reagents in **29.055** and following:

(**a**) *Iodine soln.*—0.05*N*. Dissolve 13.5 g pure I in soln of 24 g KI in 200 ml H_2O, and dil. to 2 L. Do not stdze.

(**b**) *Sodium sulfite soln.*—1%. Dissolve 1 g Na_2SO_3 in 100 ml H_2O. Make fresh daily.

(**c**) *Bromocresol green soln.*—Dissolve 150 mg bromocresol green in 100 ml H_2O.

29.111 DETERMINATION

Pipet 20 ml fraction *A* into 200 ml vol. flask. Add 40 ml 0.05*N* I soln by pipet, then with vigorous mixing add 25 ml 0.1*N* NaOH over 30 sec. period, and immediately place flask in 18 ±0.1° water bath. Exactly 10 min. after alkali addn, add 5 ml 1*N* H_2SO_4 and remove from bath. Exactly neutralize I with Na_2SO_3 soln, using 2 drops starch soln near endpoint. Back-titr. with dil. I if necessary. Add 5 drops bromocresol green and exactly neutralize soln with 1*N* NaOH; then make just acid to indicator. Dil. to vol. and det. reducing value of 5 ml aliquots by Shaffer-Somogyi method, **29.056**.

Make duplicate blanks and detns. Deduct titrn from that of blank and calc. fructose:

$$\% \text{ fructose} = \frac{500[(\text{titer} \times 0.1150) + 0.0915] \times 100}{\text{mg sample}}$$

Fructose correction for dextrose detn = f.c. = [(titer × 0.1150) + 0.0915] × 40. Bracketed quantity is mg fructose in 5 ml aliquot, valid between 0.5 and 1.75 mg fructose.

Dextrose

29.112 REAGENTS

Sodium thiosulfate soln.—0.05*N*. Prep. from stdzed stock 0.1000*N* soln, **42.035**.

29.113 DETERMINATION

Pipet 20 ml fraction *A* into duplicate 250 ml erlenmeyers. Evap. to dryness on steam bath in air current. Add 20 ml H_2O, pipet in 20 ml 0.05*N* I, and add 25 ml 0.1*N* NaOH slowly, as in fructose detn. Immediately place in 18 ±0.1° H_2O bath. Exactly 10 min. after alkali addn, add 5 ml 2*N* H_2SO_4, remove from bath, and titr. with 0.05*N* $Na_2S_2O_3$, using starch soln.

Make duplicate blanks, using H_2O, subtract titrn value from that of blank, and calc. dextrose:

$$\% \text{ dextrose} = \frac{56.275[(\text{titer} - 0.01215) \times \text{f.c.}] \times 100}{\text{mg sample}},$$

where f.c. = fructose correction from fructose detn. Equation is valid over range 10–50 mg dextrose in 20 ml. In presence of dextrose, 1 mg fructose requires 0.01215 ml 0.05*N* $Na_2S_2O_3$, in range 15–60 mg fructose.

Reducing Disaccharides as Maltose

29.114 DETERMINATION

Pipet 5 ml aliquots of fraction *B* into 25 × 200 mm test tubes, and det. reducing value as in **29.056**, except boil tubes 30 min. Value for 15 min. H_2O blank may be used here. Calc. % reducing disaccharides as maltose:

$$\% \text{ "maltose"} = \frac{50[(\text{titer} \times 0.2264) + 0.075] \times 100}{\text{mg sample}}$$

Maltose correction for sucrose detn = m.c. = maltose titer × 0.92. Bracketed quantity is mg maltose in 5 ml aliquot, valid between 0.15 to 3.80 mg maltose. Reducing value of maltose at 15 min. is 92% of final value.

Sucrose

29.115 DETERMINATION

Pipet 25 ml fraction *B* into 50 ml vol. flask. Add 5 ml 6*N* HCl and 5 ml H_2O. Mix, let stand in 60° H_2O bath 17 min., cool, and neutralize to bromocresol green with 5*N* NaOH (103 g/500 ml). (Polyethylene squeeze bottle is excellent for holding and delivering alkali.) Adjust to acid color of indicator, using 2*N* H_2SO_4 to correct over-run. Dil. to vol. and det. reducing value of 5 ml aliquots by Shaffer-Somogyi detn, **29.056**. Subtract titrn from blank, and calc. sucrose by reference to curve constructed from following table:

Sucrose in 5 ml Aliquot Oxidized, mg	*0.005N $Na_2S_2O_3$ Required, ml*
0.255	1.75
0.502	3.95
1.004	8.72
1.260	11.28

From curve obtain S_1 = sucrose equiv. to maltose correction, **29.114**, and S_2 = sucrose equiv. of sucrose titer.

$$\% \text{ sucrose} = \frac{50(2S_2 - S_1) \times 100}{\text{mg sample}}$$

Higher Sugars or "Dextrin"

29.116 DETERMINATION

Pipet 25 ml aliquots of fraction *C* into 50 ml vol. flasks. Add 5 ml 6*N* HCl and 5 ml H_2O, and heat in boiling H_2O bath 45 min. Cool, neutralize as for sucrose, dil. to vol., and det. reducing value by Shaffer-Somogyi detn, **29.056.** Subtract titrn value from blank and obtain dextrose equiv. from curve constructed from following table:

dextrose, mg	*Titer, ml*
0.05	0.20
0.10	0.60
0.25	1.85
0.50	4.00
1.00	8.50
2.00	17.60

$$\% \text{ higher sugars} = \frac{40(\text{dextrose equiv.}) \times 100}{\text{mg sample}}$$

NOTES: For most accurate work, Shaffer-Somogyi values must check within 0.04 ml. Calibration of entire procedures, including column, using known synthetic mixts of dextrose, levulose, sucrose, maltose, and raffinose (corrected for moisture) is recommended for critical work. Sugar: ml $Na_2S_2O_3$ relations: [(titer $\times$ 0.1150) + 0.0915] in equation for % fructose, and [(titer $\times$ 0.2264) + 0.075] in equation for % maltose, are obtained by analyzing known mixts of dextrose and fructose for fructose, and known amounts of maltose, resp., by the method.

Efficiency of column sepn may be checked by paper chromatography of fractions *A*, *B*, and *C* as in 29.119.

Commercial Glucose

Qualitative Test (36)—Official

29.117 REAGENT

Aniline-diphenylamine chromogenic reagent.—Dissolve 500 mg diphenylamine.HCl and 0.55 ml redistd aniline in 50 ml acetone. Add 5 ml 85% H_3PO_4. Prep. fresh daily.

29.118 PREPARATION OF SAMPLE

Dil. sample with equal vol. H_2O. To 0.5 ml in small centrifuge tube (11 $\times$ 100 mm test tube) add 4 ml absolute alcohol, shake, and centrifuge. Decant clear or slightly cloudy supernatant, dissolve ppt in 0.5 ml H_2O, reppt with 4 ml absolute alcohol, and centrifuge. Decant, and dissolve ppt in 0.1 ml H_2O. Apply 2 microliters to origin of paper chromatogram, as well as control spots of authentic honey and/or honeydew and corn sirup treated as above.

29.119 CHROMATOGRAPHY

For details of performing paper chromatography see **24.100.**

Ascending or descending is satisfactory. Suitable solvent for latter is *n-propanol-EtOAc-H_2O*, 7:1:2. Equilibrate 45 min. and irrigate at least 40 hr, letting solvent drip from serrated lower edge of paper. For shorter ascending use (ca 6 hr) roll paper into cylinder, staple edges, and set in cylindrical jar, using *isoamyl alcohol-pyridine-H_2O*, 7:7:6. To obtain increased resolution, dry paper and repeat irrigation 1 or more times.

Irrigate with suitable solvent, remove, and dry paper chromatogram. Dip in chromogenic reagent, let acetone evap., and heat at 85–95° ca 5–8 min. until control spots of corn sirup treated as above give blue color. Honey or honeydew sample contg 5% of commercial glucose shows series of blue maltodextrin spots of low R_F, converging to origin. Honey and honeydew dextrin spots are distinctly brown or gray, not blue. If paper is heated excessively, both honey dextrin spots and maltodextrin spots will approach same shade of gray.

29.120 *Quantitative Estimation—Procedure*

Approx. detn can be made by Browne's formula as follows: Multiply difference in polarizations of invert soln at 20° and 87°, **29.101,** by 77 and divide this product by % invert sugar found in sample after inversion. Multiply quotient by 100 and divide product by 26.7 to obtain % honey in sample; 100% − % honey = % glucose (*37*).

Commercial Invert Sugar (*38*)

Resorcinol Test (39)—Procedure

29.121 REAGENT

Resorcinol soln.—Dissolve 1 g resublimed resorcinol in 100 ml HCl (sp. gr. 1.18–1.19).

29.122 TEST

Place 10 ml 50% honey soln in test tube and add 5 ml ether. Shake gently and let stand until ether layer is clear. Transfer 2 ml of clear ether soln to small test tube and add large drop of recently prepd resorcinol soln. Shake and note color. Cherry red color appearing within 1 min. indicates presence of commercial invert sugar. Yellow to salmon shades have no significance.

Aniline Chloride Test (40)—Procedure

29.123 REAGENT

Aniline chloride soln.—To 100 ml aniline add 30 ml HCl (1+3).

29.124 TEST

Place 5 g sample in porcelain dish and add, while stirring, 2.5 ml recently prepd aniline reagent. In presence of commercial invert sugar, within 1 min. reagent assumes orange-red color turning dark red. Yellow to salmon shades have no significance.

NOTE: Resorcinol test and aniline chloride test, when negative, may not be regarded as conclusive evidence of absence of commercial invert sugar sirup in honey.

Diastatic Activity of Honey (*41*)—First Action

29.125 PRINCIPLE

Buffered sol. starch-honey soln is incubated and time required to reach specified end point is detd by photoelec. photometer. Results are expressed as ml 1% starch hydrolyzed by enzyme in 1 g honey in 1 hr.

29.126 APPARATUS

(a) *Reaction vessel.*—Attach sealed side-arm, 18×60 mm, to 18×175 mm test tube. Lower side of side-arm is attached 100 mm from bottom of tube, making 45° angle with lower portion of tube.

(b) *Photoelectric photometer.*—Equipped with 660 mμ red filter, or 600 mμ interference filter.

29.127 REAGENTS

(a) *Iodine stock soln.*—Dissolve 8.80 g resublimed I in 30–40 ml H_2O contg 22.0 g KI, and dil. to 1 L with H_2O.

(b) *Iodine soln.*—0.0007*N*. Dissolve 20 g KI and 5.00 ml I soln, (a), in H_2O and dil. to 500 ml. Prep. fresh every second day.

(c) *Acetate buffer soln.*—pH 5.3 (1.59*M*). Dissolve 87 g $NaOAc.3H_2O$ in 400 ml H_2O, add ca 10.5 ml HOAc in H_2O, and dil. to 500 ml. Adjust pH to 5.30 with NaOAc or HOAc, if necessary.

(d) *Sodium chloride soln.*—0.5*M*. Dissolve 14.5 g NaCl in H_2O and dil. to 500 ml.

(e) *Starch soln.*—Weigh 2.000 g sol. starch (Pfanstiehl, reagent grade, Improved Lintner Method or equiv.) and mix with 90 ml H_2O in 250 ml erlenmeyer. Rapidly bring to boil, swirling soln as much as possible. Reduce heat and boil gently 3 min., cover, and let cool to room temp. Transfer to 100 ml vol. flask and dil. to vol. Observe procedure closely to limit variation in starch-I absorbance values of blank.

29.128 STANDARDIZATION

Pipet 5 ml starch soln into 10 ml H_2O and mix well. Pipet 1 ml of this soln into several 50 ml graduated cylinders contg 10 ml of the dil. I soln. Mix well, and det. H_2O diln necessary to produce absorbance value of 0.760±0.02 in photometer test tube (or cell) combination to be used. This is std diln for starch prepn used. Repeat when changing starch source.

29.129 DETERMINATION

Weigh 5 g sample into 20 ml beaker, dissolve in 10–15 ml H_2O and 2.5 ml buffer soln, and transfer to 25 ml vol. flask contg 1.5 ml NaCl soln. Dil. to vol. (Soln must be buffered before adding to NaCl soln.)

Pipet 5 ml starch soln into side arm of reaction tube and 10 ml sample soln into bottom of tube, with care not to mix. Place tube in H_2O bath 15 min. at 40±0.2°; then mix contents by tilting tube back and forth several times. Start stopwatch. At 5 min., remove 1 ml aliquot with 1 ml serological pipet and add rapidly to 10.00 ml dil. I soln in 50 ml graduated cylinder. Mix, dil. to previously detd vol., and det. absorbance in photoelec. photometer. Note time from mixing of starch and honey to addn of aliquot to I as reaction time. (Place 1 ml pipet in reaction tube for reuse when later aliquots are taken.) Continue taking 1 ml aliquots at intervals until absorbance value of <0.235 is obtained.

The 5 min. value gives an approximation of end point as follows:

Absorbance	*End Point, min.*
0.7	>25
0.65	20–25
0.6	15–18
0.55	11–13
0.5	9–10
0.45	7–8

29.130 CALCULATION

Plot absorbance against time on rectilinear paper; draw straight line thru starting absorbance and as many points as possible. From graph det. time dild reaction-I mixt. reaches absorbance of 0.235. Divide 300 by this time to obtain diastase no. (DN).

NOTES: A 5-min. reading is sufficient for approximating end point of sample of high DN (>35) if another value is taken soon enough to obtain absorbance of ca 0.20. For accurate results, repeat detn, taking samples each min. from start. With samples of low DN, another reading at 10 min. will permit prediction of end point by plotting the data. No addnl readings need be taken till within few min. of endpoint. Only 2 such readings are needed. The 5 min. value will not accurately predict low DN.

29.131 **Free Acid—Official**

Dissolve 10 g sample in 75 ml CO_2-free H_2O and mix thoroly. Titr. with 0.1*N* NaOH, using 4–6

drops carefully neutralized phthln. (Pink color of indicator should persist at least 10 sec.) Det. blank on H_2O and indicator, and correct titrn. For honeys grading "amber" or darker, reduce wt sample to 5–6 g. Express result as ml 0.1*N* NaOH required to neutralize 100 g sample.

Titrn by pH meter to pH 8.30 (corrected) may be substituted for titrn with phthln.

MAPLE PRODUCTS (*42*)

29.132 Preparation of Sample—Procedure

(a) Maple Sirup

(1) *For solids determination.*—If sample contains no sugar crystals or suspended matter, decant enough clear sirup for detn. If sugar crystals are present, redissolve by heating at ca 50°. If suspended matter is present, filter sample thru cotton wool.

(2) *For other determinations.*—If sugar crystals are present, redissolve by heating. If other sediment is present, distribute it evenly thru sirup by shaking. Transfer ca 100 ml sirup, with its suspended sediment, to casserole or beaker, add ¼ vol. H_2O, and evap. over flame. When temp. of boiling sirup approaches 104°, draw small quantity into thin-wall, ca 1 ml pipet, and cool to room temp. in running H_2O. Wipe outside of pipet, let possibly dild sirup in point escape, transfer some of remaining sirup to refractometer, and det. solids content of cooled sirup. Repeat procedure from time to time until reading is obtained corresponding to 64.5% solids ($n_{20} = 1.4521$), or to such other value as in experience of analyst will give filtered sirup of 65.0% solids. Filter sirup thru filter that will let the 100 ml pass within 5 min. and adjust filtrate to $65.0 \pm 0.5\%$ solids (refractometric) by thoro mixing with appropriate quantity of H_2O.

(b) Maple Sugar and Other Solid or Semi-Solid Products

(1) *For moisture and solids determination.*—Grind in mortar, if necessary, and mix thoroly.

(2) *For other determinations.*—To prep. sirup dissolve ca 100 g sample in 150 ml hot H_2O, boil until temp. approaches 104°, and complete prepn of resulting sirup as in (*a*)(2), beginning "draw small quantity into thin-wall, ca 1 ml pipet, . . ."

Moisture or Solids—Official

29.133 *Maple Sugar*

Proceed as in **29.005**, or preferably **29.006**, using sample prepd as in **29.132**(*b*)(1).

29.134 *Maple Sirup, Maple Cream, etc.*

Proceed as in **29.006**, **29.007**, or **29.011**, using prepd sample, **29.132**(*a*)(1).

29.135 Ash—Official

Using 5 g prepd sirup, **29.132**(*a*)(2) or (*b*)(2), proceed as in **29.012** or **29.013**.

29.136 Soluble and Insoluble Ash—Official—*See* **29.015**

29.137 Alkalinity of Soluble Ash—Official—*See* **29.016**

29.138 Alkalinity of Insoluble Ash—Official—*See* **29.017**

29.139 Alkalinity of Total Ash—Official

Add alkalinities of sol. and insol. portions from **29.137** and **29.138**.

Polarization—Official

29.140 *Direct Polarization—See* **29.025**(a)

29.141 *Invert Polarization*

(**a**) *At 20°.*—Proceed as in **29.025**(**b**) or (**c**) or **29.026**(**b**) or (**c**).

(**b**) *At 87°.*—Proceed as in **29.034**.

29.142 Sucrose—Polarimetric Methods—Official

Proceed as in **29.025** or **29.026**, or calc. from results of **29.140** and **29.141**(a), using appropriate formula from **29.025** or **29.026**.

Sucrose—Chemical Methods

29.143 *By Reducing Sugars Before and After Inversion—Official—See* **29.032**

29.144 Reducing Sugars as Invert Sugar—Official

(**a**) *Before inversion.*—Proceed as in **29.037** or **29.039**, using aliquot of soln used for direct polarization, **29.140**. If soln is clarified, only neutral $Pb(OAc)_2$ soln may be used, and excess of Pb must be removed with dry $Na_2C_2O_4$.

(**b**) *After inversion.*—Proceed as in **29.037** or **29.039**, using aliquot of soln used for invert polarization **29.141**(a). If soln is clarified, only neutral $Pb(OAc)_2$ soln may be used, and excess of Pb must be removed with dry $Na_2C_2O_4$.

29.145 Commercial Glucose—Procedure—*See* **29.033** or **29.034**

Lead Number

Canadian Lead Number (Fowler Modification) (43)—Official

29.146 REAGENT

Basic lead acetate std soln.—Activate litharge by heating 2.5–3 hr to 650–670° in muffle (cooled product should be lemon color). In 500 ml erlenmeyer provided with reflux condenser, boil 80 g

neutral $Pb(OAc)_2.3H_2O$ and 40 g of the freshly activated litharge with 250 g H_2O 45 min. Cool, filter off any residue, and dil. with recently boiled H_2O to density of 1.25 at 20°.

29.147 DETERMINATION

Weigh sample contg 25 g dry matter, transfer to 100 ml vol. flask, and dil. to mark at 20°, or use soln in which conductivity value has been detd, **29.153.** Pipet 20 ml into large test tube, add 2 ml of the std basic $PbO(Ac)_2$ soln, cork, and let stand 2 hr.

Filter with suction thru 25 ml tared gooch, with asbestos mat at least 3 mm thick. When nearly all liquid has run thru, fill crucible with cold H_2O. Repeat to total of 4 washings, taking care to prevent formation of fissures in ppt by keeping it covered with H_2O and avoiding too great suction. Dry at 100°, weigh, and multiply wt by 20.

NOTE: Filtration may be facilitated, and necessity of keeping ppt in crucible covered with H_2O obviated, by stirring weighed quantity (0.5 g or less) of dry asbestos fiber with ppt and supernatant shortly before filtration.

Winton Lead Number (44)—Official

29.148 REAGENT

Dilute basic lead acetate std soln.—To measured vol. reagent, **29.146,** add 4 vols H_2O, and filter. Conduct blank with each set of detns.

29.149 DETERMINATION OF LEAD IN THE BLANK

Transfer 25 ml of the dil. std basic $Pb(OAc)_2$ soln to 100 ml vol. flask, add few drops HOAc, and dil. to mark with H_2O. Shake, and det. $PbSO_4$ in 10 ml of the soln as in **29.150.** Use of HOAc is imperative in order to retain all Pb in soln when reagent is dild with H_2O.

29.150 DETERMINATION

Transfer 25 g sample with H_2O to 100 ml vol. flask. Add 25 ml of the dil. std basic $Pb(OAc)_2$ soln and shake. Fill to mark, shake, and let stand at least 3 hr before filtering. Pipet 10 ml clear filtrate into 250 ml beaker, add 40 ml H_2O and 1 ml H_2SO_4, shake, and add 100 ml alcohol. Let stand overnight, filter on weighed gooch, wash with alcohol, dry in 100° oven, and ignite in muffle at 550°, or over Bunsen burner, placing crucible in larger crucible, applying heat gradually at first and heating to barely visible red color of outside crucible. Cool and weigh. Subtract wt $PbSO_4$ so found from wt $PbSO_4$ found in blank, **29.149,** and multiply by factor 27.33. Use of this factor gives Pb number directly (without various calcns otherwise required).

Conductivity Value (*45*)—Official

29.151 APPARATUS

(a) *Conductivity cell.*—Made of resistance glass with platinized Pt electrodes firmly fixed and adequately protected from displacement. These electrodes may be sealed into vessel into which soln under examination may be run and subsequently drawn off (*Zerban type*), or attached to support so that they can be lowered into cylinder (or 100 ml beaker) contg the soln (dipping type). Provide cell with thermometer graduated in 0.1° and covering 20–30°, and place bulb in immediate vicinity of electrodes. Cell constant should be ca 0.15.

(b) *Galvanometer or microphone hummer (or induction coil) and sensitive telephone receiver.*

(c) *Source of current.*—Dry or storage cells if hummer or induction coil is used; 110 volt alternating current if galvanometer is used.

(d) *Resistances of 10 and 100 ohms.*—Should be fixed and accurate.

(e) *Slide wire or Wheatstone bridge.*

(f) *Device for control of temp. of cell to within ±0.1°.*—Constant temp. bath or vessel into which H_2O of suitable temp. may be run so as to adjust cell contents to 25°.

29.152 DETERMINATION OF CELL CONSTANT

Prep. solns of 0.3728 and 0.7456 g dry KCl in H_2O, which offers resistance of at least 25,000 ohms in the cell, and dil. to mark at 20–25° in 500 ml vol. flasks. Fill cell with the more dil. (0.01*M*) soln, adjust to 25±0.1°, measure resistance, and multiply number of ohms by 141.2 (sp. conductivity of 0.01*M* KCl). Rinse with stronger (0.02*M*) soln, fill cell with this soln, measure its resistance at 25°, and multiply by 276.1 (sp. conductivity of 0.02*M* KCl). Average 2 results.

29.153 DETERMINATION

Weigh sample contg 25 g dry matter, transfer to 100 ml vol. flask with warm H_2O of same quality as that used in detn of cell constant, cool to 25°, dil. to mark, and measure resistance in cell at 25±0.1°. Divide cell constant by number of ohms found.

conductivity value

= cell constant × 10^5/sp. conductivity (ohms).

Malic Acid (*46*)—Official

29.154 APPARATUS

(a) *Ion exchange columns.*—Std wall Pyrex glass tubing, 10 mm i.d.×30 cm long, with 5 cm capillary tip.

(b) *Spectrophotometer.*—Suitable for measuring

absorption at 390 mμ, with matched 1 cm cells or matched test tubes.

29.155 REAGENTS

(a) *Ion exchange resins.*—(1) *Cation exchanger.* —Dowex-50 (60–80 mesh). (2) *Anion exchanger.* —Amberlite IRA 400 (60–80 mesh) or Amberlite CG 400, Type I (100–200 mesh).

(b) *Ammonium carbonate soln.*—0.25*N*. Dissolve 14.26 g $(NH_4)_2CO_3.H_2O$ in enough H_2O to make 1 L.

(c) *Ammonium carbonate soln.*—1.0*N*. Dissolve 57.05 g $(NH_4)_2CO_3.H_2O$ in enough H_2O to make 1 L.

(d) *Sodium carbonate soln.*—1.0*N*. Dissolve 5.3 g Na_2CO_3 in enough H_2O to make 100 ml.

(e) *Hydrochloric acid soln.*—5%. Dil. 12 ml HCl with 88 ml H_2O.

(f) *2,7-Naphthalenediol.*—1 g dissolved in 100 ml H_2SO_4.

(g) *Malic acid std soln.*—Dry Eastman Kodak white label L-malic acid 18 hr at 40°. Dissolve 0.2000 g in 500 ml H_2O. Dil. known vol. of this soln (ca 10 ml) to 100 ml so that final soln gives absorbance, *A*, after reaction with color reagent as in detn, of 0.2–0.8.

29.156 PREPARATION OF ION EXCHANGE COLUMNS

For each column add enough H_2O to 10 ml dry resin to make thin slurry and pour slurry into column contg small plug of glass wool. Let H_2O drain to level of settled resin and wash with 2 ml portions H_2O to condition resins. To cation exchange resin (Dowex-50) add three or four 10 ml portions 5% HCl, letting acid drain to top of resin between each addn. Wash resin free of acid with 10 ml portions H_2O until effluent gives no test for chlorides. (Approx. 4 bed vols of H_2O are required.)

Treat anion exchanger (IRA 400) resin with three or four 10 ml portions 5% NaOH soln, draining liquid to top of resin between addns. Remove excess of alkali with H_2O by washing with 10 ml portions until effluent gives negative alkali test with indicator paper. Transform resin into carbonate form by addn of three or four 10 ml portions 1.0*N* Na_2CO_3 soln. Wash free of carbonate with 10 ml portions H_2O until effluent is neutral to indicator test paper. Mount conditioned columns vertically with cation resin column directly above anion resin column, connecting tubes with 1-hole rubber stopper mounted in top of anion column. No stopcocks are required; close packing of fine resins prevents liquid from draining below surface of resins. Any portion of resin that becomes dry will be inactivated.

29.157 SEPARATION OF MALIC ACID

Transfer ca 10 ml sirup sample to tared 100 ml vol. flask and weigh to ±0.0002 g. Dil. to mark with H_2O and transfer aliquot contg 6–20 mg malic acid (ca 15 ml) to cation exchange resin and let eluate pass onto anion exchange resin. Wash cation resin (upper column) with three 10 ml portions H_2O, again letting effluent pass directly onto anion resin. Remove upper column and wash anion resin column with three 10 ml portions H_2O to remove sugars and any loosely held acids present that might interfere with test. Elute column with five 10 ml portions 0.25*N* $(NH_4)_2CO_3$ to quantitatively remove all of the glycolic, glyceric, or lactic acids possibly present in original test soln. Elute malic acid from anion resin with five 10 ml portions 1*N* $(NH_4)_2CO_3$; after 45–48 ml eluate collects in 250 ml vol. flask, remove flask and dil. to vol. with H_2O.

29.158 DETERMINATION

Transfer 1 ml of the malic acid-$(NH_4)_2CO^3$ eluate to 18×150 mm culture tube and slowly add 6 ml 96% H_2SO_4 from buret, adding first 2 ml down walls of tube to avoid excessive evolution of CO_2. Add 0.1 ml of the 2,7-naphthalenediol reagent and mix thoroly. Cap tubes with metal culture tube closures and heat in boiling H_2O bath (deepfat fryer is satisfactory) 25 min. to develop color. Cool tubes, and measure absorbances of colored solns within 30 min. in 1 cm absorption cell at 390 mμ against blank of 1 ml H_2O, 6 ml H_2SO_4, and 0.1 ml reagent also heated 25 min. in boiling H_2O bath.

Color developed follows Beer's law in which $a = A/Cb$, where a is absorptivity, C is concn in mg/ml, and b is cell thickness. Absorptivity may vary from day to day because of differences in blank; therefore a must be established daily with duplicate portions of fresh std malic acid soln. Calc. a from absorbance A at 390 mμ of colored soln resulting from reaction of soln of std malic acid and color reagent. Calc. amount of malic acid in sample from: $C = (A/ab) \times$ diln factor. Express value for malic acid in maple sirup in terms of std density (65.5° Brix) sirup.

SUGAR BEETS

Sucrose

29.159 *Hot Water Digestion Method I. (47)—First Action*

Pass sample (usually in form of cossettes) thru meat grinder fitted with plate having ⅛″ perforations and mix thoroly. Weigh 26 g prepd sample and rinse into 201.0 ml Kohlrausch flask, using ca 100 ml H_2O. Place flask under good vac. 5–10 min. to remove air, carefully avoiding mechanical loss when vac. is first applied. Add H_2O to ca 175

ml, and digest in H_2O bath at 80°, supporting flask so that body is entirely immersed but is not in contact with heating element. Remove flask 2 or 3 times during digestion, swirl contents, and after each agitation wash down pulp adhering to walls of flask with little H_2O at 80°.

After exactly 30 min. digestion, fill flask to within 2–5 ml of mark with H_2O at 80° and continue digestion exactly 10 min. longer. Cool to room temp. in H_2O bath. Add 6 ml basic $Pb(OAc)_2$ soln, **29.021**(a), and H_2O to fill to mark. (Previous addns of H_2O and reagents should be so adjusted that not >4 ml H_2O is required to make to vol.) Mix well by shaking, let stand 5 min., shake again, and filter. After soln stands near saccharimeter at least 5 min., polarize in 400 mm glass tube. If vol. adjustment and polariscopic observation are made at 20°, reading gives % sucrose directly; if at other temps, apply formula in **29.022**(a).

NOTES: The 1 ml >200 ml vol. is the detd vol. of marc for beets grown in Colorado and neighboring states. It should be detd for other localities. Beets of abnormally low purity may require 8–10 ml basic $Pb(OAc)_2$ soln for clarification. If trouble is experienced with foam, flask may be put under vac. second time after cooling, or few drops of ether or 1 drop of *amyl alcohol* may be added before soln is dild to vol.

29.160 *Hot Water Digestion Method II. (48)—First Action*

Use Ni-plated sheet Fe vessels, 11 cm high, 6 cm body diam., and 4 cm mouth diam.; use stoppers covered with Sn foil to fit.

Weigh 26 g prepd beet pulp, **29.159**, on watch glass (small enough to go into neck of beaker) and transfer to metal beaker; add 177 ml dil. basic $Pb(OAc)_2$ soln (5 parts basic $Pb(OAc)_2$ soln, **29.021**(a), to 100 parts H_2O); shake, and stopper lightly. Submerge beaker in H_2O bath 30 min. at 75–80°, shaking intermittently. When all air is expelled (generally after 5 min.), tighten stopper. After 30 min., shake, cool to std temp., filter, add drop HOAc to filtrate, and polarize in 400 mm tube. Reading is % sugar in beet pulp.

STARCH CONVERSION PRODUCTS—FIRST ACTION

29.161 **Preparation of Sample**—*See* **29.001**

Moisture

29.162 *Method I.*

(Applicable to refined corn sugars)—*See* **29.006**

Method II. (49)

(Applicable to corn sirups and crude corn sugars)

29.163 APPARATUS

(a) *Filter paper.*—Strip of Whatman No. 1 filter paper, 4.375×50 cm.

(b) *Separator.*—Corrugated strip of phosphor-bronze, No. 36 B&S, 1.25×40 cm.

(c) *Weighing bottle.*—Medium form, 40×65 mm, with ₮ 40/20 stopper.

29.164 DETERMINATION

Place separator on filter paper strip and roll together to form cylinder ca 30 mm in diam., fasten with paper clip, and place in weighing bottle. Dry in air oven at 100° ca 6 hr. Cool and weigh. Remove coil and weigh ca 1 g sirup in weighing bottle, add 1 or 2 ml H_2O, and mix, using heat if necessary. Replace coil, shaking until all soln is absorbed on paper. Dry to constant wt in vac. oven at 100° for corn sirups or 70° for crude corn sugars (ca 6 hr).

Method III. (50)

(Applicable to corn sirups and crude corn sugars)

29.165 MATERIAL AND APPARATUS

(a) *Diatomaceous earth (Filter Cel).*—Preferably analytical grade. If commercial grade is used, wash with HCl, then with H_2O to remove acid, and dry in oven at ca 105°. (Material should give no test for acid when moistened.)

(b) *Moisture dish.*—Al dish 25 mm high×75 mm diam., with cover.

(c) *Pestle.*—Flat-end glass stirring rod ca 60 mm long.

29.166 DETERMINATION

Place 10 g of the Filter-Cel in moisture dish contg pestle and dry to constant wt. Weigh ca 5 g corn sirup or sugar in nickel scoop, dil. with ca 5 ml H_2O, and add to Filter-Cel. Wash scoop with three 2 ml portions H_2O and add washings to Filter-Cel. Thoroly incorporate soln with Filter-Cel by means of pestle, yielding *damp* workable mass. Dry to constant wt in vac. oven at 100° for corn sirup or 70° for crude corn sugars.

Dry Substance

Method I.—By Hydrometer (51)

29.167 APPARATUS

(a) *Water bath.*—Insulated H_2O bath with stirrer and thermostatic control, held at 60°.

(b) *Cylinders.*—Pyrex, $15\times2\frac{1}{4}''$, without lip.

(c) *Stopper seal.*—Consisting of 2 rubber stoppers that fit snugly into cylinder, sepd on metal rod by ca 3″. Rod is fixed in lower stopper but does not extend thru it. Top stopper is free to move on rod, altho tight enough to maintain predetd position, preventing evapn during heating.

(d) *Baumé hydrometers.*—Streamlined type, modulus 145, stdzd at 15.56° with range 35–45° Bé. in 0.1° Bé.; length over-all 12–13″; body diam. 0.77–0.79″; scale length 147–155 mm.

29.168 DETERMINATION

Fill cylinder with sirup to within 4″ of top, taking care that sides are free from sirup. Seal cylinder with stopper seal, placing bottom stopper within ½″ of sirup surface and closing cylinder with top stopper. Immerse cylinder in H_2O bath at 60° (140° F) so that level of sirup is ca 2″ below level of H_2O. Immerse hydrometer in H_2O bath. When sirup in cylinder is free of air and has reached temp. of bath (ca 90 min.), raise cylinder until surface of sirup is at eye level. Remove stopper seal and insert previously dried hydrometer. After ca 10 min. read hydrometer. To obtain commercial Baumé, add 1° Bé. to observed reading of hydrometer:

Commercial Baumé = Bé. (140°F/60°F) + 1° Bé.

Det. corresponding dry substance from **29.170**.

29.169 *Method II.—By Refractometer* (*52*)

(Applicable only to liquid samples contg no undissolved solids)

Det. refractometer reading at 45°. Circulate H_2O thru jackets of refractometer long enough to let temp. of prisms and of sample reach equilibrium, continuing circulation during observation, and taking care that temp. is held constant. From **29.171** obtain commercial Baumé corresponding to observed refractive index. From **29.170** obtain corresponding dry substance.

29.172 **Ash**—*See* **29.012**

(For most corn sugars and sirups it is unnecessary to re-ash)

29.173 **Acidity**

Weigh 50 g sample, dissolve in 200 ml H_2O, and titr. with 0.1*N* NaOH, using phthln, to faint pink end point. (This corresponds to electrometric pH of 8.3.) Calc. acidity as HCl.

29.174 **Hydrogen-Ion Concentration**

Prep. soln of sample contg 40% total solids and det. pH electrometrically. (Buffer capacity of product is normally such that no special provision need be made with regard to H_2O used for diln. If glass electrode is used, stdze against reference buffer within 1.0 of detd pH.)

29.175 **Nitrogen**—*See* **2.036**

29.176 **Total Reducing Sugars**

(a) *Lane-Eynon General Volumetric Method.—See* **29.037**.

(b) *Munson-Walker General Method.—See* **29.039**.

Dextrose (Steinhoff Method)

Zerban-Sattler Modification (*53*)

29.177 REAGENTS

(a) *Soxhlet modification of Fehling copper soln.—See* **29.035**(a).

29.170 *Commercial table for dry substance in corn sirup and corn sugar sirup*

(Commercial Baumé = Bé. 140°F/60°F + 1° Bé.)

COMMERCIAL BAUMÉ	DEXTROSE EQUIVALENT AND ASH							
	30.00 0.28	42.00 0.28	55.00 0.30	82.00 0.41	87.00 0.61	89.00 0.61	91.2 0.61	90.7 1.22
	DRY SUBSTANCE (PER CENT)							
40.00	73.66	74.39	75.16	76.82	77.12	77.24	77.37	77.10
41.00	75.58	76.34	77.14	78.86	79.18	79.30	79.44	79.17
42.00	77.51	78.30	79.13	80.92	81.25	81.38	81.52	81.25
43.00	79.45	80.27	81.14	83.00	83.35	83.48	83.63	83.33
44.00	81.39	82.25	83.17	85.10	85.46	85.60	85.75	85.44
45.00	83.36	84.25	85.20	87.21	87.58	87.72	87.88	87.56
46.00	85.34	86.26	87.26	89.33	89.71	89.86	90.03	89.69
47.00	87.33	88.29	89.34	91.47	91.87	92.03	92.21	91.84

29.171 *Commercial table of refractive indices of corn sirups and corn sugar sirups at 45°C*

(Commercial Baumé = Bé. 140°F/60°F + 1° Bé.)

COMMERCIAL BAUMÉ	DEXTROSE EQUIVALENT AND ASH									
	30.00 0.28	35.00 0.28	42.00 0.28	45.00 0.28	50.00 0.30	55.00 0.30	60.00 0.30	65.00 0.30	82.00 0.41	89.00 0.61
	REFRACTIVE INDEX AT 45°									
40.00	1.4774	1.4773	1.4771	1.4770	1.4769	1.4768	1.4767	1.4766	1.4762	1.4760
41.00	1.4825	1.4824	1.4822	1.4821	1.4820	1.4820	1.4818	1.4817	1.4813	1.4811
42.00	1.4878	1.4877	1.4875	1.4874	1.4873	1.4873	1.4871	1.4869	1.4865	1.4863
43.00	1.4933	1.4931	1.4929	1.4928	1.4927	1.4926	1.4924	1.4923	1.4919	1.4916
44.00	1.4986	1.4985	1.4983	1.4982	1.4981	1.4980	1.4978	1.4977	1.4973	1.4971
45.00	1.5041	1.5040	1.5038	1.5037	1.5036	1.5036	1.5034	1.5033	1.5029	1.5027
46.00	1.5098	1.5097	1.5095	1.5094	1.5093	1.5092	1.5090	1.5089	1.5085	1.5082
47.00	1.5155	1.5154	1.5152	1.5151	1.5150	1.5149	1.5148	1.5147	1.5143	1.5142

(b) *Sodium acetate soln.*—Dissolve 500 g $NaOAc.3H_2O$ in ca 800 ml hot H_2O, cool, and dil. to 1 L.

(c) *Potassium iodide-iodate soln.*—Dissolve 5.4 g KIO_3 and 60 g KI in H_2O, add 0.25 g NaOH dissolved in little H_2O, and dil. to 1 L.

(d) *Sulfuric acid.*—Approx. 2*N*. Dil. 57 ml H_2SO_4 to 1 L.

(e) *Saturated potassium oxalate soln.*—Dissolve 165 g $K_2C_2O_4.H_2O$ in 500 ml hot H_2O, and cool.

(f) *Sodium thiosulfate std soln.*—0.1*N*. Dissolve ca 25 g $Na_2S_2O_3.5H_2O$ in H_2O and dil. to 1 L. Stdze against pure Cu as in **29.041.**

(g) *Sugar soln.*—Dissolve quantity of sample contg ca 10 g solids in H_2O and dil. to 1 L.

29.178 DETERMINATION

Transfer 10 ml Soxhlet soln, 20 ml NaOAc soln, 10 ml sugar soln, and 10 ml H_2O to 250 ml erlenmeyer. Mix contents, close flask with rubber stopper provided with Bunsen valve, and immerse in briskly boiling H_2O bath exactly 20 min. Immerse in cold running H_2O, venting valve to prevent boiling caused by vac. Cool, add 25 ml $KI–KIO_3$ soln by pipet and mix by gentle shaking. Add rapidly 40 ml 2*N* H_2SO_4 from graduated cylinder; then add 20 ml $K_2C_2O_4$ soln from graduated cylinder. Shake until ppt completely dissolves, and titr. excess I with 0.1*N* $Na_2S_2O_3$.

Det. blank, substituting H_2O for sugar soln. Difference between titer of blank and that of sample is direct measure of Cu_2O pptd. From **29.179** obtain dextrose equiv. corresponding to titer of 0.1*N* $Na_2S_2O_3$.

Correction for reducing effect of maltose.—If maltose is present, correct observed titer of 0.1*N* $Na_2S_2O_3$ for reducing effect of maltose by subtracting correction obtained from **29.179** by interpolation.

Sichert-Bleyer Modification (54)

29.180 REAGENTS

(a) *Ferric ammonium sulfate soln.*—Dissolve 120 g $Fe_2(SO_4)_3.(NH_4)_2SO_4.24H_2O$ and 100 ml H_2SO_4 in H_2O and dil. to 1 L.

(b) *Potassium permanganate soln.*—0.1*N*. Dissolve ca 3.16 g $KMnO_4$ in H_2O and dil. to 1 L. *See also* **29.043**(a).

29.181 STANDARDIZATION

To obtain factor for 0.1*N* $KMnO_4$ make analysis as in **29.182** on 10 ml soln contg 50 mg pure dextrose. From **29.183** titer of 15.38 ml corresponds to 50 mg dextrose; 15.38 divided by titer obtained gives correction factor for the $KMnO_4$ soln. Multiply all titers by this factor before referring to **29.183.** Redet. factor each day analyses are made.

29.182 DETERMINATION

Transfer 10 ml Soxhlet soln, **29.035**(a), 20 ml NaOAc soln, **29.177**(b), 10 ml sugar soln prepd as in **29.177**(g), and 10 ml H_2O to 250 ml erlenmeyer. Mix contents and close flask with rubber stopper provided with Bunsen valve. Immerse in boiling H_2O bath exactly 20 min. Filter Cu_2O ppt thru gooch prepd as in **29.038,** and wash flask and crucible 3 times with hot H_2O. (It is not necessary to remove all ppt from flask.)

Transfer asbestos mat and crucible to 150 ml beaker marked at 60 ml. Wash flask with exactly 20 ml of the $FeNH_4(SO_4)_2$ soln in 3 portions and transfer quantitatively to beaker contg crucible. (All ppt must be dissolved.) Finally wash flask and crucible with hot H_2O and remove crucible. Add hot H_2O to 60 ml mark. Heat soln to boiling on hot plate, let stand 3 min., and titr. with the 0.1*N* $KMnO_4$. Addn of 1 ml H_3PO_4 toward end of titrn facilitates reading of end point. Pink-gray end point persists ca 20 sec. Multiply titer by factor and obtain mg dextrose from **29.183.**

29.179 *Zerban-Sattler table for determination of dextrose with copper acetate reagent**

TITER	DEXTROSE (MG)										MALTOSE CORRECTIONS (SUBTRACT FROM OBSERVED TITER) MALTOSE PRESENT (MG)		
	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	200	100	50
10	25.7	26.0	26.3	26.6	26.9	27.2	27.5	27.8	28.1	28.4	2.5	1.4	0.6
11	28.7	29.0	29.3	29.6	29.9	30.3	30.6	30.9	31.2	31.5	2.3	1.2	.4
12	31.8	32.2	32.5	32.9	33.2	33.6	34.0	34.3	34.7	35.0	2.2	1.1	.4
13	35.4	35.8	36.1	36.5	36.8	37.2	37.6	37.9	38.3	38.6	2.0	1.0	.3
14	39.0	39.4	39.9	40.3	40.7	41.2	41.6	42.0	42.4	42.9	1.9	1.0	.3
15	43.3	43.8	44.2	44.7	45.1	45.6	46.1	46.5	47.0	47.4	1.8	1.0	.3
16	47.9	48.4	49.0	49.5	50.1	50.6	51.1	51.7	52.2	52.8	1.7	1.0	.3
17	53.3	53.9	54.5	55.2	55.8	56.4	57.0	57.6	58.3	58.9	1.6	0.9	3
18	59.5	60.2	60.9	61.6	62.3	63.1	63.8	64.5	65.2	65.9	1.4	.8	.3
19	66.6	67.4	68.2	69.0	69.8	70.6	71.4	72.2	73.0	73.8	1.2	.7	.3
20	74.6	75.6	76.5	77.5	78.4	79.4	80.3	81.3	82.2	83.2	1.0	.6	.2
21	84.1	85.2	86.3	87.4	88.5	89.6	90.6	91.7	92.8	93.9	0.6	.4	.2
22	95.0										.4	.3	.1

* Table may be interpolated for each .01 ml, but should *not* be extrapolated.

29.183 *Sichert-Bleyer table for determination of dextrose**

TITER 0.1N PER-MAN-GANATE	DEXTROSE (MG)									
	0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
ml										
10	26.5	26.8	27.1	27.4	27.8	28.1	28.4	28.7	29.0	29.3
11	29.7	30.0	30.4	30.7	31.1	31.5	31.8	32.2	32.6	32.9
12	33.3	33.7	34.1	34.5	34.9	35.4	35.8	36.2	36.6	37.0
13	37.4	37.9	38.4	38.8	39.3	39.8	40.3	40.7	41.2	41.7
14	42.2	42.7	43.2	43.8	44.3	44.9	45.4	46.0	46.5	47.0
15	47.6	48.2	48.8	49.4	50.1	50.7	51.3	51.9	52.5	53.2
16	53.8	54.5	55.2	55.9	56.6	57.3	58.0	58.7	59.4	60.2
17	60.9	61.7	62.5	63.3	64.1	64.9	65.7	66.5	67.4	68.2
18	69.0	69.9	70.9	71.9	72.8	73.8	74.8	75.7	76.7	77.6
19	78.6	79.6	80.7	81.7	82.7	83.7	84.8	85.8	86.8	87.8
20	88.9	90.0	91.2	92.3	93.5	94.7	96.0	97.2	98.5	99.7

* Table may be interpolated for each .01 ml, but should *not* be extrapolated.

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30. Vegetable Products, Processed

CANNED PRODUCTS

30.001 Drained Weight

Weigh full can, open, and pour entire contents on round sieve with No. 8 std screen (wire diam. 0.84 mm; size of opening 2.38 mm) (*1*). Use 8″ diam. sieve for No. 3 or smaller can, and 12″ for larger cans. Without shifting product, incline sieve so as to facilitate drainage. Drain 2 min., weigh either drained solids or free liquid direct, and weigh dry empty can. From wts thus obtained det. % liquid and % solid contents.

30.002 Preparation of Sample—Procedure

(**a**) *Products composed of solid and liquid portions.*—If only solid portion is required for analysis or examination, thoroly grind drained vegetables in mortar or food chopper. If composite of solid and liquid portion is required, thoroly grind entire contents of can in mortar or food chopper. In all cases, thoroly mix portion used and store balance in g-s. container. Unless analysis is to be completed in reasonably short time, det. H_2O in portion of sample prepd as above, and to prevent decomposition dry remainder, grind, mix thoroly, and store in g-s. container. (Second H_2O detn is required in this procedure.)

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(**b**) *Comminuted products (tomato juice, tomato catsup, strained vegetables)* (*2*).—Shake unopened container thoroly to incorporate any sediment. Transfer entire contents to large glass or porcelain dish, and mix thoroly, continuing stirring at least 1 min. Transfer well-mixed sample to g-s. container and shake or stir thoroly each time before removing portions for analysis.

30.003 Total Solids—Official

Weigh, into flat-bottom dish, portion of sample of such size that dry residue will be not <9 mg nor >12 mg/sq. cm of drying surface. Distribute thinly in even layer over bottom of dish, dilg with H_2O if necessary to facilitate distribution. Place in vac. oven at 70° with release cock left partly open so that degree of vac. is not >450 mm of Hg and H_2O evolved is carried off rapidly. Dry air admitted thru release cock by bubbling thru H_2SO_4. After 1 hr examine dishes and remove from oven any in which material has reached apparent dryness. Continue this removal of dishes with dried material at subsequent 30 min. intervals. After material in all dishes has reached apparent dryness, return dishes to oven, nearly close release cock so that ca 2 bubbles of air/sec. are admitted thru the H_2SO_4, and dry 4 hr at 70° at pressure not >100 mm.

30.004 Insoluble Solids (*3*)—Official

Wash 20 g sample repeatedly with hot H_2O, centrifuging after each addn of H_2O and pouring clear supernatant thru weighed filter paper on büchner. (Filter used is 1 of 2 such papers dried 2 hr at 100° and weighed in covered dish. Use second paper, if necessary, when first becomes clogged.) After 4 or 5 washings, transfer remaining insol. matter to filter, dry in uncovered dish 2 hr at 100°, cover, cool in desiccator, and weigh.

30.005 Soluble Solids—Official

% total solids − % insol. solids = % sol. solids.

+

30.006 Specific Gravity (*4*)—Official

(Applicable to comminuted tomato products)

Det. sp. gr. at 20/20°, using National Canners Association tomato pulp sp. gr. bottle. Clean and calibrate bottle at 20° as in **9.010**, but since bottle is not provided with cap, strike off excess H_2O with straight edge, wipe bottle dry, and weigh immediately. Cool sample to 16–18°, fill bottle with the pulp, and centrifuge 1 min. at ca 1000 rpm. Add enough pulp to fill bottle to top and centrifuge again. Remove bottle and take temp. of pulp, inserting thermometer so that no air is introduced. When temp. is just 20°, remove thermometer, add enough pulp at same temp. to have bottle slightly over full, and strike off even with straight edge. Clean outside of bottle and weigh at once to nearest 0.01 g. Sp. gr. = wt pulp in bottle ÷ wt H_2O at 20° that bottle holds.

30.007 Ash—Official—*See* 29.012 or 29.013

30.008 Alkalinity of Ash—Official

Proceed as in **20.017.** Express result as ml 1*N* acid required to neutralize ash from 100 g sample.

Sodium Chloride—Official

30.009 *Method I.*

Proceed as in **6.066** or **6.068,** using HNO_3 soln of ash, **6.065.** Calc. and report result as % NaCl.

30.010 *Method II. (Rapid Method)* (*5*)

Weigh ca 5 g material, transfer with 80% alcohol to 100 ml vol. flask, and add enough 80% alcohol to give vol. of ca 50 ml. Shake well to suspend all insol. material. Add 1 ml HNO_3 and with pipet add excess of 0.1*N* $AgNO_3$ soln. Dil. to 100 ml with alcohol. Transfer mixt. to centrifuge bottle and centrifuge 5 min. at ca 1800 rpm. Pipet 50 ml supernatant into 300 ml erlenmeyer, add 2 ml satd $FeNH_4(SO_4)_2$ soln and 2 ml HNO_3, and titr. to permanent light brown with 0.1*N* NH_4CNS. Divide ml 0.1*N* $AgNO_3$ used by 2 and subtract ml NH_4CNS soln used. Multiply difference by 0.005845 to obtain g NaCl present.

30.011 Reducing Sugars Before Inversion—Official

Weigh 20 g sample into 200 ml vol. flask, dil. with ca 100 ml H_2O, clarify with slight excess of neutral $Pb(OAc)_2$ soln, **29.021(d)**, dil. to mark, and filter. Remove excess of Pb with anhyd. Na_2SO_4 or with dry K oxalate. Filter, and det. reducing sugars as in **29.039**. Express result as % invert sugar.

30.012 Reducing Sugars After Inversion—Official

Transfer 50 ml filtrate, **30.011**, to 100 ml vol. flask, add 5 ml HCl, and let stand overnight, as in **29.026(c)**. Nearly neutralize with NaOH soln, cool, dil. to mark, and det. reducing sugars in aliquot as in **29.039**. Express result as % invert sugar.

30.013 Sucrose—Official—*See* 29.032.

30.014 Total Acids—Official

Proceed as in **20.040** or **20.041**, using 5 g sample. Express result as ml 1*N* alkali required to neutralize 100 g sample.

30.015 Alcohol-Insoluble Solids in Canned Peas (*6*)—Official

Pour sample on No. 8 screen, using 8″ size for containers of <3 lbs net wt and 12″ for larger quantities. Spread peas evenly and let drain. Transfer peas to white pan and remove any foreign material. Add vol. H_2O equal to double vol. original sample.

Pour peas back on screen, spreading evenly, tilt screen as much as possible without shifting peas, and drain 2 min. With cloth wipe surplus moisture from lower surface of screen. Grind drained peas in food chopper until cotyledons are reduced to smooth homogeneous paste, stir, and weigh 20 g ground material into 600 ml beaker. Add 300 ml 80% alcohol, stir, cover beaker, and bring to boil. Simmer slowly 30 min.

Fit into büchner filter paper of appropriate size (previously prepd by drying in flat-bottom dish 2 hr at temp. of boiling H_2O, covering with tight-fit cover, cooling in desiccator, and weighing at once). Apply suction and transfer contents of beaker to büchner so as to avoid running over edge of paper. Suck dry and wash material on on filter with 80% alcohol until washings are clear and colorless.

Transfer paper and alcohol-insol. solids to dish used in prepn of paper, dry uncovered 2 hr at temp. of boiling H_2O, place cover on dish, cool in desiccator, and weigh at once. From this wt deduct wt dish, cover, and paper. Calc. % by wt of alcohol-insol. solids.

30.016 Field Corn in Canned Mixtures of Field and Sweet Corn (*7*)—Official

Empty contents of No. 2 can, or representative equiv. portion of larger can, into large beaker and remove liquor and debris from fragments of kernels by flotation with cold H_2O. Place upon flat plate all kernels to which outer seed coat is still attached, mix thoroly, and quarter to ca 400 pieces. Harden selected pieces in alcohol and quarter again to obtain ca 100 fragments.

Cut each fragment thru with section razor or knife and avoid contamination of fragments with dextrin by washing and drying instrument after each cut. With dissecting needle remove portion ca $\frac{1}{16}$″ in diam. from uncontaminated interior of each kernel and place pieces in separate depressions of white spot plate. Cover each piece with *freshly prepd I stain* (0.2 g I, 1.5 g KI in 100 ml H_2O) and let stand 10 min. Brown cloud disseminates from portions of sweet corn owing to presence of dextrin, while soln surrounding the field corn remains clear and field corn portions are blue-black and sharply outlined. Crush apparent field corn portions to insure absence of dextrin and count those found to contain none.

Use care in interpreting results, because kernels of immature sweet corn do not contain enough dextrin to produce dense brown color characteristic of more mature sweet corn. In case of doubt, report as field corn only those kernels having firm texture and showing no brown color with I soln on 2 confirmatory tests. Calc. % field corn from total number kernels examined.

Lactic Acid—Official

30.017 PREPARATION OF SOLUTION

Weigh 50 g ground and mixed sample into tared centrifuge bottle and add 100 ml H_2O. Make acid to Congo red paper with 1*N* H_2SO_4. Adjust wt of contents of bottle to 200 g by addn of H_2O, shake vigorously, and centrifuge. Decant supernatant and weigh 100 g into 100–110 ml vol. flask. Dil. to 110 ml mark with H_2O, shake, and pipet 50 ml into continuous extractor (Fig. 26, p. 187).

Add 0.5 ml H_2SO_4 (1+1) and 2 ml 20% $Na_2WO_4.2H_2O$ soln, and proceed as in **15.012.**

If sample contains HOAc added in course of manufacture (*e.g.*, catsup), transfer extd material, after evapn of ether, to beaker, add ca 50 ml H_2O, and evap. to 20 ml. Again add 50 ml H_2O and evap. to 20 ml. Neutralize with satd $Ba(OH)_2$ soln and proceed as in **15.013.**

FROZEN VEGETABLES

30.018 Catalase—First Action—*See* 17.003

Aldehydes as Acetaldehyde (*8*)—First Action

30.019 APPARATUS

Steam distilling apparatus.—Consisting of 500 ml Kjeldahl flask, spray trap, and condenser, preferably all-glass with ₮ joints (Catalog No. J-1356, Scientific Glass Apparatus Co., Bloomfield, N. J., is satisfactory). App. with rubber stoppers and connections is suitable if satisfactory blanks are obtained. (Some rubber yields negative blanks.)

30.020 REAGENTS

(**a**) *Sodium thiosulfate std soln.*—0.05*N*. Prep. as in **42.035,** using 12.5 g $Na_2S_2O_3.5H_2O$, and stdze as in **42.036.** 1 ml 0.05*N* $Na_2S_2O_3$ = 1.1 mg AcH.

(**b**) *Iodine std soln.*—0.05–0.0515*N*. Prep. as in **4.004**(**b**), stdze against $Na_2S_2O_3$ soln, (**a**), and adjust concn, if necessary.

(**c**) *Sodium bisulfite std soln.*—0.05*N* in ca 10% alcohol. Dissolve 2.60 g $NaHSO_3$ in 500 ml H_2O in 1 L vol. flask. Add 100 ml alcohol, mix, and dil. to vol. with H_2O. Stdze against I soln, (**b**), and adjust concn, if necessary, so as to be slightly weaker than that of the I soln, but not <0.0485*N*. Prep. fresh daily.

(**d**) *Aldehyde-free alcohol.*—Use alcohol contg <2 ppm AcH; if greater, purify as in **19.054**(**a**). Det. aldehydes as in **30.021,** using 300 ml freshly boiled and cooled H_2O and 35 ml of the alcohol, beginning "Pipet in 25 ml of the $NaHSO_3$ soln . . ." Det. blank similarly but without alcohol. Difference in titrns ×31 = ppm aldehydes as AcH.

30.021 DETERMINATION

Sharply strike package of frozen vegetable on surface or edge of table to break up block. Mix well and grind ca 150 g thru food chopper.

Transfer 50 g sample to Kjeldahl flask with ca 150 ml H_2O. Add 1–2 drops *DC Antifoam*, or equiv., and steam distill into 500 ml erlenmeyer, immersed in ice bath and contg 100 ml chilled, freshly boiled H_2O to cover end of delivery tube. Collect ca 200 ml within 12–15 min. To distillate add 35 ml of the aldehyde-free alcohol. Pipet in 25 ml of the $NaHSO_3$ soln and let stand 30 min., shaking occasionally. Pipet in 25 ml of the I soln, and titr. with the $Na_2S_2O_3$ soln, using 5 ml starch indicator, **17.001**(**g**). Det. blank on 300 ml freshly boiled and cooled H_2O. Difference in ml×1.1 = mg AcH; mg AcH×1000/wt sample = ppm.

SELECTED REFERENCES

(*1*) U. S. Dept. Commerce, NBS Specifications for Sieves, U. S. Standard Sieve Series.
(*2*) U. S. Dept. Agr. Bur. Chem. Bull. **162,** p. 124.
(*3*) Ibid. **152,** 118(1911).
(*4*) N.C.A. Bull. **27-*L***, Revised 1950, p. 26; J. Assoc. Offic. Agr. Chemists **19,** 254(1936).
(*5*) J. Assoc. Offic. Agr. Chemists **22,** 765(1939); **23,** 353(1940); **24,** 424(1941); **25,** 466(1942).
(*6*) Ibid. **21,** 90(1938); **22,** 87(1939).
(*7*) Ibid. **11,** 136(1928); **12,** 39(1929); **15,** 167(1932).
(*8*) Ibid. **39,** 282(1956).

31. Waters, Mineral, and Salt

MINERAL WATERS*

31.001 Specific Gravity—Official

Det. sp. gr. at 20/20°, using pycnometer, as in **9.011**.

31.002 Total Solids—Official

Thoroly shake sample, and pipet 100 ml unfiltered sample into weighed Pt dish. If sample contains much suspended matter, shake, pour rapidly into 100 ml graduate, and transfer without delay to weighed Pt dish. Evap. to dryness and heat to constant wt at 100°.

31.003 Solids in Solution—Official

Let sample stand until all sediment settles and filter if necessary to secure perfectly clear liquid. (Occasionally, clear filtrate can be obtained only by use of alumina cream, **29.021(b)**, but this should be avoided if possible.) Evap. 100–250 ml to dryness in weighed Pt dish. Heat to constant wt at 100°.

31.004 Ignited Residue—Official

Ignite residue from **31.002** at 525–550° in muffle or over burner until dish shows dull red glow and ash is white or nearly so. Note any odor or change in color produced during ignition. Record wt ignited residue and calc. loss on ignition.

Nitrogen in Form of Nitrate (*1*)

Phenoldisulfonic Acid Method—Official

(For water of low Cl content)

31.005 REAGENTS

(a) *Phenoldisulfonic acid soln.*—Dissolve 25 g pure white phenol in 150 ml H_2SO_4, add 75 ml fuming H_2SO_4 (13–15% SO_3), and heat 2 hr at 100°.

(b) *Nitrate std soln.*—Dissolve 0.607 g pure $NaNO_3$ in 1 L NO_3-free H_2O. Evap. 50 ml of this soln to dryness in porcelain dish; when cool, treat with 2 ml of the phenoldisulfonic acid soln, grind and stir with glass rod to insure intimate contact, and dil. to 500 ml. 1 ml = 0.01 mg N (0.044 mg NO_3). (This soln is permanent.) Prep. stds for comparison by adding NH_4OH to measured vols of the std soln in 100 ml Nessler tubes as in detn.

(c) *Silver sulfate std soln.*—Dissolve 4.397 g Ag_2SO_4, NO_3-free, in 1 L H_2O. 1 ml = 1 mg Cl.

31.006 DETERMINATION

To 100 ml sample, or quantity contg 0.05 mg or less of N, add enough std Ag_2SO_4 soln to ppt all but ca 0.5 mg of the Cl. Heat to boiling and let settle, or add little alumina cream, **29.021(b)**, filter, and wash with small quantities of hot H_2O. Evap. filtrate to dryness in porcelain dish on steam bath; when cool, treat with 2 ml of the phenoldisulfonic acid soln as in **31.005(b)**. Dil. with H_2O and slowly add NH_4OH until max. color is developed. Filter if necessary, transfer to 100 ml Nessler tubes, and compare with stds in usual manner. Record result as mg N or nitrate/L.

Reduction Method (2)—Official

(For water of high Cl content)

31.007 REAGENTS

(a) *Aluminum foil.*—Purest obtainable. Cut into ca 10 cm strips, weighing ca 0.5 g each.

(b) *Nessler reagent.*—Dissolve 143 g NaOH in 950 ml H_2O and filter thru asbestos. Add 50 g red HgI_2 to filtrate and dil. with H_2O to 1 L. Mix thoroly, let settle, and use supernatant.

(c) *Sodium hydroxide soln.*—Dissolve 250 g pure NaOH in 1250 ml H_2O. Add 2 or 3 strips of the Al foil and let stand ca 12 hr. Conc. soln to 1 L by boiling.

(d) *Ammonium chloride std soln.*—Dissolve 3.818 g NH_4Cl in NH_3-free H_2O and dil. to 1 L. Then dil. 10 ml of this soln to 1 L (1 ml = 0.01 mg N).

31.008 DETERMINATION

To 100 ml sample, or quantity contg 0.1 mg or less of nitrate N in 300 ml casserole, add 2 ml of the NaOH soln and conc. by boiling to ca $\frac{1}{3}$ original vol. Transfer to 100 ml test tube, using N-free H_2O, and dil., if necessary, to ca 75 ml. Prep. blank (preferably several blanks, since N impurity in Al is often distributed unevenly) by placing ca 75 ml N-free H_2O and 2 ml of the NaOH soln in 100 ml test tube. Place strip of the Al foil in each tube. Close ends of test tubes with rubber stoppers connected by bent glass tubes to other test tubes contg ca 50 ml slightly acidified NH_3-free H_2O. (These latter tubes serve as traps to prevent escape of NH_3 and at same time permit

* The official methods for nitrogen, **2.036** and **2.037**, conform with the recommendations made by the Joint Committee on Uniformity of Methods for Water Examination, 1959.

free evolution of H.) Let sample and blank stand at room temp. 12 hr or until reduction is complete.

Nesslerize contents of trap tubes by addn of 2 ml Nessler reagent. If high in NH_3, indicating frothing over of sample, discard detn. Disregard traps if they contain only 0.01–0.02 mg each of N as NH_3.

Transfer sample and blank to distn flasks, using 250 ml NH_3-free H_2O for each; distill at rate of ca 1 tubeful in 10 min., into 50 ml Nessler tubes until NH_3 ceases to be given off (4 or 5 tubes are usually enough). Add to each tube 2 ml of the Nessler reagent and let stand 10 min. From small buret measure into Nessler tubes definite quantities of the std NH_4Cl soln, dil. contents of each tube to 50 ml with NH_3-free H_2O, add 2 ml of the Nessler reagent, and compare depth of color with Nesslerized distillate. Report as mg N or nitrate/L.

Chloride—Official

31.009 REAGENTS

(a) *Potassium chromate indicator.*—Dissolve 5 g K_2CrO_4 in H_2O, add satd $AgNO_3$ soln until slight permanent red ppt forms, filter, and dil. to 100 ml.

(b) *Silver nitrate std soln.*—Dissolve 4.791 g $AgNO_3$ in H_2O and dil. to 1 L. 1 ml = 1 mg Cl. Stdze as in **42.027.**

31.010 DETERMINATION

To 100 ml sample add few drops of phthln. If pink color appears, titr. CO_3 thus indicated to bicarbonate with 0.05*N* H_2SO_4. If sample is acid to Me orange, add 0.05*N* Na_2CO_3 to neutralize acidity. Add 1 ml K_2CrO_4 indicator and titr. with the std $AgNO_3$ soln. Correct for quantity of $AgNO_3$ soln necessary to give, in 100 ml Cl-free H_2O with 1 ml of the K_2CrO_4 soln, shade obtained at end of titrn of sample. If iodides and bromides are found in interfering quantities, make equiv. correction.

If Cl is present in very small quantities, conc. 500 or 1000 ml in porcelain dish to 100 ml, rub down sides of dish carefully, add 1 ml K_2CrO_4 indicator, and titr. with the std $AgNO_3$ soln. If enough Cl is present in 100 ml of the water to consume >25 ml std $AgNO_3$ soln, det. by pptn and weigh AgCl as in **6.066.**

Fluorides (*3*)—Official

31.011 REAGENTS

(a) *Sodium fluoride stock soln.*—Dissolve 2.22 g NaF (min. purity 98%) in 1 L H_2O. (1 ml = 1 mg F.)

(b) *Sodium fluoride std soln.*—Dil. 10 ml stock soln (a) to 1 L. (1 ml = 0.01 mg F.)

(c) *Thorium nitrate soln.*—Dissolve 0.25 g $Th(NO_3)_4.12H_2O$ or 0.2 g $Th(NO_3)_4.4H_2O$ in 1 L H_2O.

(d) *Alizarin red indicator.*—0.01% aq. soln Na alizarin sulfonate (alizarin red S).

(e) *Hydrochloric acid.*—Exactly 0.05*N*.

(f) *Sodium hydroxide soln.*—Exactly 0.05*N*.

(g) *Hydroxylamine hydrochloride soln.*—1.0 g /100 ml.

31.012 APPARATUS

(a) *Claisen flask.*—250 ml.

(b) *Nessler tubes.*—6 long-form 50 ml tubes with double optically plane disks fused to tubes. Match tubes for length and test for optical similarity as follows: Add ca 40 ml H_2O, 1 ml of the indicator, 2 ml of the HCl, and H_2O to mark on tube. Then to 1 tube add such quantity of the $Th(NO_3)_4$ soln that, after making to mark and mixing, color is barely changed to faint pink. Note quantity of $Th(NO_3)_4$ soln used. Add same quantity of $Th(NO_3)_4$ soln to each of remaining 5 tubes. Reject tubes showing detectable differences in shade or intensity.

See also **24.027.**

31.013 PREPARATION OF SAMPLE

If sample has odor of H_2S, oxidize with 0.1 ml 30% H_2O_2 soln before evapn.

Place 100 ml sample in porcelain or Pt dish, make alk. to phthln with 10% NaOH soln (avoid excess), and evap. to 20 ml over burner at temp. just below b.p. During evapn keep sample alk. by adding small quantities of the NaOH soln from time to time. Transfer the 20 ml evapd sample to Claisen flask contg glass beads or boiling tube previously rinsed with boiling 10% NaOH soln to eliminate all traces of gelatinous SiO_2 accumulating in flask.

Place flask contg sample on asbestos board ($6'' \times 6'' \times \frac{1}{4}''$ with $1''$ center hole) over burner adjusted for medium flame. Close straight neck of flask with 2 hole rubber stopper thru which pass thermometer and stem of small separator with outlet constricted to 2 mm diam. (Adjust thermometer and outlet tube of separator to extend almost to bottom of flask.) Close other neck of flask with solid rubber stopper. (Alternatively, all-glass distn assembly may be used.)

Connect flask with H_2O condenser, add 20 ml 60% $HClO_4$ to flask via evapg dish and separator; then add quantity of *satd $AgClO_4$ soln* that will ppt chlorides (detd previously by titrn with std $AgNO_3$ soln), and distill at $132 \pm 3°$, adding H_2O dropwise thru separator to maintain temp. during distn. Collect nearly 200 ml distillate. Dil. to vol. (200 ml) and mix well. To det. acidity use 40 ml distillate, add 1 ml of the indicator, mix thoroly, and note ml of the 0.05*N* NaOH required for neutralization.

Repeat prepn and distn, using 100 ml H_2O in place of sample, to det. blank.

31.014 DETERMINATION

Prep. one std, one color comparison tube, and one or more sample tubes as follows:

(**a**) *Color comparison tube.*—To 40 ml H_2O add 2 ml of the 0.05*N* HCl, 1 ml of the alizarin red indicator, 1 ml of the $NH_2OH.HCl$ soln, and enough $Th(NO_3)_4$ soln to give faint but definite pink end point. Compare all end point colors with this color.

(**b**) *Sample tube.*—To sample tube contg 40 ml distillate add 1 ml of the indicator, 1 ml of the $NH_2OH.HCl$ soln, and such quantity of 0.05*N* HCl that total quantity of acid in tube (acidity previously detd plus quantity of 0.05*N* HCl added) equals 2 ml 0.05*N* HCl. Dil. to vol. and mix. If in preliminary acidity detn it is found that the 40 ml distillate requires >2 ml of the NaOH soln for neutralization, do not add the HCl soln to the sample tube, but add to the std tube same quantity of acid as was found present in sample tube. If 40 ml distillate requires >5 ml 0.05*N* NaOH, repeat distn under conditions favorable to low acidity. From 10 ml buret, graduated to 0.05 ml, add the $Th(NO_3)_4$ soln with frequent mixing until faint pink appears, comparable to comparison tube (**a**). Note vol. $Th(NO_3)_4$ soln used.

(**c**) *Standard tube.*—To std tube contg 40 ml H_2O add 1 ml of the indicator, 1 ml of the $NH_2OH.HCl$ soln, and 2 ml or more of the 0.05*N* HCl, as was required in sample tube in (**b**). If aliquot chosen for detn already contains 2–5 ml 0.05*N* acid, add exactly same quantity to std tube. Add exactly same quantity of the $Th(NO_3)_4$ soln as was added to sample tube. To std tube (now more highly colored than sample tube) add the std NaF soln from 10 ml buret with mixing until color matches that of sample tube. Dil. contents of both std and sample tubes to same vol. Mix soln in each tube and let all air bubbles escape before making color comparisons. Check end point by adding 1–2 drops of the NaF soln to std tube. Distinct color change should develop.

31.015 CALCULATION

Subtract ml NaF soln required by blank from ml NaF soln required by sample.

$$\frac{\text{ml NaF soln} \times \text{ml total distillate} \times 10}{\text{ml aliquot titrd} \times \text{wt sample taken}} = \text{F (ppm)}.$$

EXAMPLE: 100 ml sample, evapd and distd to 200 ml, of which 40 ml aliquot corresponds to 5 ml of the NaF soln, gives:

$$\frac{5 \times 200 \times 10}{40 \times 100} = 2.5 \text{ F (ppm)}.$$

Hydrogen Sulfide (*4*)—Official

31.016 REAGENTS

(**a**) *Iodine soln.*—0.02*N*. Dissolve 10 g KI in 1 L flask, using as little H_2O as possible. Add 2.54 g resublimed I and dissolve by shaking. Dil. to mark with H_2O. Stdze against $Na_2S_2O_3$ soln that has been recently stdzd against $K_2Cr_2O_7$ soln.

(**b**) *Iodine soln.*—0.01*N*. Mix equal vols (**a**) and boiled H_2O. Stdze against $Na_2S_2O_3$ soln as in (**a**).

31.017 DETERMINATION

Siphon quantity of sample to graduated vessel and add few drops phthln. If alk., add HCl until pink color of indicator disappears. Add starch indicator, **4.004**(**f**), and with careful stirring, titr. with I soln, **31.016**(a) or (**b**), to permanent blue. Correct for quantity of I soln needed to give equally blue color. From corrected quantity of I soln used, calc. approx. quantity of H_2S present.

For accurate detns siphon 100–500 ml sample, according to quantity of H_2S present, into graduated vessel, keeping outlet of siphon in liquid. Add immediately enough HCl, calcd from the approx. detn to make neutral to phthln. Mix carefully with bent glass rod, and without delay add ca 0.5 ml less I reagent (**a**) or (**b**) than is needed to combine with H_2S present.

Add 5 ml starch indicator **4.004**(**f**), and finish titrn by adding the I soln dropwise with stirring to permanent blue. Correct for quantity of I soln needed to give equally blue color when same quantity of starch soln is added to ca equal vol. boiled H_2O. If possible, make several detns and take av. Stdze reagents (**a**) and (**b**) frequently.

31.018 Carbonate and Bicarbonate—Official

To 100 ml sample add few drops phthln, and if pink color is produced, titr. with 0.05*N* HCl or H_2SO_4, adding drop every 2–3 sec. until color disappears. Multiply buret reading by factor 3 to obtain mg CO_3 ion in 100 ml. To colorless soln from this titrn, or to original soln if no color is produced with phthln, add 1 or 2 drops Me orange, continue titrn without refilling buret, and note total reading. If CO_3 is absent, multiply total buret reading by factor 3.05 to obtain value of HCO_3 ion in mg/100 ml. If CO_3 is present, multiply reading with phthln by 2 and subtract from total reading of buret. Multiply difference by 3.05 to obtain HCO_3 ion in mg/100 ml. Express results as mg/L.

31.019 Silica—Official

Make preliminary examination, using 100–250 ml sample, to det. approx. quantity of Ca and Mg

present, in order to det. quantity of sample to be evapd for final analysis.

Evap. quantity of sample equiv. to 0.1–0.6 g CaO or 0.1–1 g $Mg_2P_2O_7$ (usually 1–5 L). Acidify the water with HCl and evap. on steam bath to dryness in Pt dish. Continue drying ca 1 hr. Thoroly moisten residue with 5–10 ml HCl. Let stand 10–15 min. and add enough H_2O to bring sol. salts into soln. Heat on steam bath until salts dissolve. Filter to remove most of the SiO_2 and wash thoroly with hot H_2O. Evap. filtrate to dryness and treat residue with 5 ml HCl and enough H_2O to dissolve sol. salts, as before. Heat, filter, and wash thoroly with hot H_2O. Designate filtrate as Soln *A*.

Transfer the two residues to Pt crucible, ignite, heat over blast lamp, and weigh. Moisten contents of crucible with few drops H_2O, add few drops H_2SO_4 and few ml HF, and evap. on steam bath under hood. Repeat treatment if all SiO_2 is not volatilized. Dry carefully on hot plate, ignite, heat over blast lamp, and weigh. Difference between the two wts is wt SiO_2. Add wt residue ($Fe_2O_3+Al_2O_3$) to that of Al_2O_3 and Fe_2O_3 obtained in **31.020.** (If residue weighs >0.5 mg, $BaSO_4$ may be present in the water. If so, make necessary correction and add to wt Fe_2O_3 and Al_2O_3 in **31.020.**)

31.020 Iron and Aluminum—Official

Conc. Soln *A*, **31.019,** to 200 ml; while still hot, add NH_4OH slowly, stirring constantly, until alk. to Me orange. Boil, filter, and wash 3 times with hot H_2O. Dissolve ppt in hot HCl (1+1). Dil. to ca 25 ml, boil, and again ppt with NH_4OH. Filter, wash thoroly with hot H_2O, dry, ignite, and weigh as Al_2O_3 and Fe_2O_3. (In presence of H_3PO_4, wt of this residue must be corrected for P_2O_5 equiv. to H_3PO_4 found in **31.033,** allowing for difference in vols of the water used for these detns.) Designate filtrate as Soln *B*.

Iron—Official

31.021 *Colorimetric Method*

(Iron <1 mg; not applicable in presence of phosphates)

Fuse, in Pt crucible, ignited ppt of Fe_2O_3 and Al_2O_3, **31.020,** with fused $KHSO_4$, dissolve in H_2O, and ppt the Fe and Al with NH_4OH. Filter, dissolve ppt on filter paper in HCl and HNO_3, dil. soln, add 3 ml 5% NH_4CNS soln, dil. to suitable vol., and compare color developed with that of calibrated color disks or stds contg known quantities of Fe treated similarly.

31.022 *Volumetric Method*

Fuse residue of Fe_2O_3 and Al_2O_3 **31.020,** in Pt crucible with ca 1 g fused $KHSO_4$. This fusion takes but few min., and must not be continued beyond time actually needed. When fusion is complete, set crucible aside and let cool. Add H_2SO_4 (1+4) and heat crucible until fused mass dissolves. Evap. on steam bath as far as possible; then heat gradually until copious fumes of SO_3 evolve. Dissolve in H_2O and let stand on steam bath. Cool, transfer to erlenmeyer, and dil. to such vol. that soln contains not >2.5% free H_2SO_4.

Pass H_2S thru soln to reduce Fe and ppt any Pt contaminating residue from fusion. (Zn may be used instead of H_2S for reducing Fe.) Filter, wash, and again pass H_2S thru soln to reduce all Fe. Expel H_2S by boiling, at same time passing current of CO_2 thru soln. Test escaping gas with $Pb(OAc)_2$ paper to confirm complete removal of H_2S. Discontinue boiling and let flask cool without discontinuing current of CO_2. Titr. reduced Fe with std $KMnO_4$ soln, 1 ml = 1 mg Fe, and calc. as Fe.

31.023 Aluminum—Official

To obtain wt Al_2O_3, in absence of phosphates, subtract from wt Fe_2O_3 and Al_2O_3, **31.020,** the Fe, **31.021** or **31.022,** calcd to Fe_2O_3. Calc. to Al.

31.024 Calcium—Official

Conc. Soln *B*, **31.020,** to 150–200 ml, and to this soln, contg equiv. of not >0.6 g CaO or 1 g $Mg_2P_2O_7$, add 1–2 g $H_2C_2O_4.2H_2O$ and enough HCl (1+1) to clear soln. Heat to boiling and neutralize with NH_4OH, stirring constantly. Add NH_4OH in slight excess and let stand 3 hr in warm place. Filter off supernatant and wash ppt once or twice by decantation with 1% $(NH_4)_2C_2O_4$ soln. Dissolve ppt in HCl (1+1), dil. to 100–200 ml, add little more $H_2C_2O_4$, and ppt as above. After letting ppt stand 3 hr, filter, wash with the 1% $(NH_4)_2C_2O_4$ soln, dry, ignite, heat over blast lamp, and weigh as CaO and SrO. From this wt subtract wt SrO equiv. to the Sr, **31.025.** Difference is wt CaO. Calc. to Ca. Designate combined filtrates and washings as Soln *C*.

As check on the CaO, evap. to dryness filtrate from the $Sr(NO_3)_2$ in **31.025,** beginning "Filter, and wash with the ether-alcohol mixt. . . . " Dissolve the $Ca(NO_3)_2$ in H_2O, ppt as oxalate, filter, wash, ignite, and weigh as CaO. $CaO \times 0.7147 = Ca$.

31.025 Strontium (*5*)—Official

Dissolve the oxides, **31.024,** in HNO_3 (1+1) and test with spectroscope for Sr. If Sr is present, transfer the HNO_3 soln to small erlenmeyer. Evap. nearly to dryness over low flame and heat 1–2 hr at 150–160° after H_2O is evapd. Break up dried material with stirring rod and add 10–15 ml mixt. of equal parts of absolute alcohol and ether to dissolve the $Ca(NO_3)_2$. Cork flask and let stand with frequent shaking 2 hr or longer. Decant soln thru 5.5 cm filter, reserving filtrate. Wash residue

several times by decantation with small portions of the ether-alcohol mixt. Dry residue and paper, and wash paper repeatedly with small portions of hot H_2O, collecting filtrate in flask contg main portion of the $Sr(NO_3)_2$ residue. Add 1 or 2 drops HNO_3 (1+1), evap., dry, pulverize, and treat with 10–15 ml of the ether-alcohol mixt. Cork flask and let stand ca 12 hr, shaking occasionally.

Filter, and wash with the ether-alcohol mixt. until few drops filtrate evapd. on watch glass leave practically no residue. Dry paper and ppt. Dissolve the $Sr(NO_3)_2$ in few ml hot H_2O. Add few drops H_2SO_4 and then add vol. alcohol equal to vol. soln and let stand 12 hr. Filter, ignite, weigh as $SrSO_4$, and calc. to Sr. Test spectroscopically for Ca and Ba. If these elements are present, det. quantity and make necessary correction.

31.026 Magnesium—Official

Conc. Soln *C*, **31.024,** to 200 ml, acidify with HCl (1+1), and add 2–3 g $(NH_4)_2HPO_4$ and enough HCl (1+1) to produce clear soln when all $(NH_4)_2HPO_4$ is dissolved. When cold, make slightly alk. with NH_4OH, stirring constantly. Add 2 ml excess of NH_4OH and let stand ca 12 hr. Filter off supernatant and wash 4 times by decantation with NH_4OH (1+10). Dissolve ppt in HCl (1+1), dil. to ca 150 ml, add little $(NH_4)_2HPO_4$, and ppt with NH_4OH as before. Let stand 12 hr, filter, wash Cl-free with NH_4OH (1+10), place in porcelain crucible, ignite, heat over blast lamp, and weigh as $Mg_2P_2O_7$. Calc. to Mg. $Mg_2P_2O_7 \times 0.21847 = Mg$.

31.027 Sulfate—Official

Make preliminary examination, using 100–250 ml sample, to det. approx. quantity of sulfates. (Alkali salts present can be approximated by calcg quantity of Na necessary to combine with excess of acids—HCl, H_2SO_4, and H_2CO_3—over the Ca and Mg.)

Take enough sample (usually 1–5 L) to yield not >1 g $BaSO_4$ and not >0.5 g mixed chlorides. Acidify with HCl (1+1), evap. to dryness in Pt dish, and remove SiO_2 by 2 evapns as in **31.019,** using not >2 ml HCl for final soln. Combine filtrate and washings from the SiO_2 detns and conc. to 150–200 ml. Heat to boiling and ppt with slight excess of 10% $BaCl_2.2H_2O$ soln, added very slowly and with constant stirring. Cover, and let stand on steam bath ca 12 hr. Filter, thoroly wash ppt of $BaSO_4$ with hot H_2O until Cl-free, dry, ignite over Bunsen burner, and weigh.

If sulfate content of sample is unusually large, proceed as far as concn of the SiO_2 filtrates, as above. Add 50 ml HCl, heat to boiling, and ppt with $BaCl_2$ soln as before. Evap. to dryness, take up in H_2O and few drops HCl, digest till ppt settles, wash by decantation, filter, ignite, and weigh. Calc. to SO_4 ion. Designate filtrate as Soln *E*.

Sodium, Potassium, and Lithium

Ether-Alcohol Method (6)—Official

31.028 PREPARATION OF MIXED CHLORIDES

Evap. Soln *E*, **31.027,** to dryness in Pt dish, and ignite residue to faint redness to remove all traces of NH_4 salts. Dissolve residue in dish in ca 200 ml H_2O and ppt with satd $Ca(OH)_2$ soln or satd $Ba(OH)_2$ soln. Boil, let stand 30 min., and filter off insol. $Mg(OH)_2$ and undissolved $Ca(OH)_2$. Thoroly wash ppt with hot H_2O and combine filtrate and washings. If ppt of Mg is large, dissolve in small quantity of HCl, evap. to dryness, take up with H_2O, and ppt as before.

Conc. the two filtrates and washings to 200–250 ml. Add NH_4OH and enough solid $(NH_4)_2CO_3$ to ppt the Ca and Ba. Let stand on steam bath 1–2 hr. Filter off supernatant, dissolve ppt in HCl, again ppt as above, and wash thoroly with hot H_2O. Evap. combined filtrates and washings to dryness and drive off NH_4 salts by gentle heat. Treat residue with H_2O, pass thru small filter, using as little wash H_2O as possible, evap. to small vol., and again ppt with 1 or 2 drops NH_4OH and 2 or 3 drops satd solns of $(NH_4)_2CO_3$ and $(NH_4)_2C_2O_4$. If any ppt appears, filter and repeat process.

Evap. filtrate to dryness and drive off all NH_4 salts by heating to faint redness in Pt dish. Treat residue with little H_2O, filter into small Pt dish, add few drops HCl (1+1), and evap. to dryness. Dry in oven; then heat to faint redness, cool in desiccator, and weigh combined chlorides of K, Na, and Li. Repeat heating to constant wt (x). Dissolve mixed chlorides in hot H_2O, filter, and wash. Return filter paper and residue to dish, dry, ignite, and weigh (y). $(x)-(y)=$ wt mixed chlorides.

31.029 DETERMINATION

Dissolve mixed chlorides, **31.028,** in min. quantity of cold H_2O (ca 1.5 ml is more than enough for 0.5 g of the salts), in tall 200 ml beaker. Add 1 drop HCl, and then add gradually 20 ml absolute alcohol, dropping alcohol into center of beaker (not on sides) while rotating soln. (NaCl and KCl should be pptd in perfectly uniform granular condition.) In similar manner add 60 ml ether (sp. gr. 0.716–0.717 at 25°) and let mixt. stand ca 5 min. or until ppt is well agglomerated and supernatant is almost clear, rotating mixt. occasionally during this period. Filter with suction thru weighed gooch into erlenmeyer, using bell jar arrangement, washing beaker thoroly with mixt. of 1 part alcohol and 5 parts ether, and collecting all ppt on gooch with aid of policeman. Wash ppt on gooch thoroly, set crucible aside,

and rinse funnel with alcohol-ether mixt. to wash any adhering Li soln into flask contg filtrate. Evap. filtrate to dryness on steam bath, using air current.

Treat residue with 10 ml absolute alcohol, warming if necessary, so that practically all residue dissolves. If slight film remains on bottom and sides of flask, remove with policeman. Then, while rotating soln in flask, add 50 ml ether (sp. gr. 0.716–0.717 at 25°), followed by 1 drop HCl. Let stand 30 min., rotating soln frequently. When fine ppt has agglomerated (only very small quantity is usually pptd), filter into tall beaker with suction thru gooch contg first ppt. Wash combined ppts with the ether-alcohol mixt., taking same precautions as in first pptn. Air-dry gooch and contents; then dry in oven, ignite gently, cool, and weigh to obtain combined wt NaCl and KCl. Reserve crucible and contents for K detn.

Evap. on steam bath ether-alcohol filtrate and washings contg the Li. Dissolve residue in little H_2O, add slight excess of H_2SO_4 (1+1), and transfer to weighed porcelain or Pt dish. Evap. as far as possible on steam bath and then gently ignite residue over flame. (By placing dish on triangle over asbestos gauze and using low flame, soln can be evapd without spattering.) Finally ignite carefully over full flame, cool, and weigh. If charring has occurred, repeat ignition with H_2SO_4. Calc. to Li, using factor 0.1263.

Remove KCl and NaCl from gooch by washing with 25–50 ml hot H_2O, using suction, and collecting filtrate in porcelain dish. Add enough Pt soln, **2.059(b)**, to convert KCl and NaCl to K_2PtCl_6 and Na_2PtCl_6, and evap. to dryness. Treat residue with 80% alcohol by vol., filter, and wash until excess of H_2PtCl_6 and Na_2PtCl_6 is removed. Dry filter and ppt, dissolve residue in hot H_2O, and transfer to weighed Pt dish. Evap. on steam bath, dry 30 min. in oven at 100°, cool, and weigh as K_2PtCl_6. Calc. to KCl, using factor 0.3067, and to K, using factor 0.1609.

Det. wt NaCl by subtracting wt KCl from wt combined KCl and NaCl. Calc. to Na, using factor 0.3934.

Barium

(It is not necessary to look for Ba if sulfate is present in appreciable quantity unless the water contains large quantity of bicarbonate or chloride, which may hold in soln small quantity of both sulfate and Ba.)

Gravimetric Method (7)—Official

31.030 REAGENTS

(a) *Ammonium dichromate soln.*—Dissolve 100 g of the SO_4-free salt in H_2O and dil. to 1 L.

(b) *Ammonium acetate soln.*—Dissolve 300 g of the salt in H_2O, neutralize with NH_4OH, and dil. to 1 L.

(c) *Dilute ammonium acetate soln.*—Dil. 20 ml (b) to 1 L.

Reaction of acetate solns should be alk. rather than acid.

31.031 DETERMINATION

Acidify 1–5 L portion of sample with HCl and conc. to ca 200 ml. (If ppt forms, filter it off and test for Ba.) Add ca 0.5 g NH_4Cl, and ppt Fe and Al with NH_4OH. Boil, filter, and wash. To filtrate add excess of the NH_4OAc soln (10 ml), keeping total vol. ca 200 ml. Heat to boiling and add, with stirring, ca 5 ml of the $(NH_4)_2Cr_2O_7$ soln. Let settle and cool. Decant clear liquid thru filter and wash ppt by decantation with the dil. NH_4OAc soln until filtrate is no longer perceptibly colored (ca 100 ml wash soln).

Place beaker under funnel, dissolve ppt on paper with warm HNO_3 (1+1), using as little as possible, and wash paper. Add little more acid to dissolve ppt in beaker, and then NH_4OH until ppt that forms no longer redissolves. Heat to boiling; add, with stirring, 10 ml of the NH_4OAc soln and 2 ml of the $(NH_4)_2Cr_2O_7$ soln; let cool slowly, and wash ppt by decantation with the dil. NH_4OAc soln. Dry the $BaCrO_4$, burn filter separately, ignite moderately to constant wt, and weigh as $BaCrO_4$. Calc. as Ba, using factor 0.5422.

31.032 *Volumetric Method—Official*

Proceed as in **31.031** thru "wash ppt by decantation with the dil. NH_4OAc soln." (after second pptn). Then proceed as follows: Dissolve ppt in ca 10 ml HCl (1+1) and hot H_2O. Wash filter, dil. soln to ca 400 ml, and add ca 50 ml freshly prepd 10% KI soln. Mix carefully and titr. liberated I after 3 or 4 min. with 0.1N $Na_2S_2O_3$. 1 ml 0.1N $Na_2S_2O_3$ = 4.579 mg Ba.

31.033 **Phosphate—Official**

Treat 500 ml of sample, or larger quantity if necessary, with ca 10 ml HNO_3 and evap. in porcelain dish nearly to dryness to drive off HCl. Treat residue with H_2O and filter if necessary. Add NH_4OH to alky and then just enough HNO_3 to restore acidity. Add some solid NH_4NO_3 and heat in 45–50° H_2O bath. Add freshly prepd molybdate soln **2.020(a)**, and keep 30 min. at 45–50°. If more than trace of yellow ppt is present, filter and wash with recently boiled and cooled H_2O until entirely acid-free. Transfer ppt and filter to beaker, add little H_2O, and beat paper and contents to pulp. Dissolve yellow ppt in small quantity of std KOH soln **2.020(b)**, add phthln, and titr. with the std acid. Ml 0.1N KOH × 0.4130 × 1000/ml sample = mg PO_4/L.

31.034 Preparation of Sample—Manganese, Iodine, Bromine, Arsenic, and Boric Acid

Evap. 0.5–2 L sample to dryness after addn of small quantities of solid Na_2CO_3. Boil residue thus obtained with H_2O, transfer to filter, and wash thoroly with hot H_2O. Use residue remaining on filter for detn of Mn. Dil. alk. filtrate to definite vol. and use for detn of I, Br, As, and H_3BO_3.

Manganese—Official

Persulfate Method

31.035 REAGENTS

(**a**) *Silver nitrate soln.*—Dissolve 2 g $AgNO_3$ in H_2O and dil. to 1 L.

(**b**) *Manganous sulfate std soln.*—Dissolve 0.2877 g pure $KMnO_4$ in ca 100 ml H_2O, acidify soln with H_2SO_4 (1+1), and slowly heat to boiling. Add slowly enough 10% $H_2C_2O_4.2H_2O$ soln to discharge color. Cool, and dil. to 1 L. 1 ml = 0.1 mg Mn.

31.036 DETERMINATION

Dissolve insol. residue **31.034,** in excess HNO_3 (1+1), evap. to dryness, treat with H_2O, and add ca 1 ml HNO_3 and little of the $AgNO_3$ soln. If ppt of AgCl appears, add more of the $AgNO_3$ soln until all Cl is pptd. Add excess of ca 10 ml of the $AgNO_3$ soln for each mg Mn present in sample. Filter, add 1 g $(NH_4)_2S_2O_8$ to filtrate, and place beaker or flask contg soln on steam bath until pink color develops (ca 20 min.). Compare color developed with stds similarly prepd by treating solns contg known quantities of the std $MnSO_4$ soln with the dil. HNO_3, $AgNO_3$ soln, and $(NH_4)_2S_2O_8$.

Bismuthate Method (8)

31.037 REAGENTS

(**a**) *Nitric acid.*—(1+4). Free from brown oxide of N by aeration.

(**b**) *Dilute sulfuric acid.*—Dil. 25 ml H_2SO_4 to 1 L with H_2O. Add enough $KMnO_4$ soln to color faintly.

(**c**) *Manganous sulfate std soln.*—Prep. as in **31.035(b)**. Soln of $KMnO_4$ may be substituted for $MnSO_4$ soln. To prep. it dissolve 0.2877 g $KMnO_4$ in H_2O and dil. to 1 L.

31.038 DETERMINATION

Remove Cl by several evapns with H_2SO_4 (1+1) from quantity of sample that contains 1 mg or less of Mn. Residue obtained in **31.034** may be substituted for fresh sample by dissolving it in excess of HNO_3 (1+4), adding the dil. H_2SO_4, and removing Cl by two or more evapns. In either case, volatilize the H_2SO_4, and ignite residue at low heat (<500°).

Dissolve in 40 ml HNO_3 (1+3), add ca 0.5 g Na bismuthate, and heat until permanganate color disappears. Add few drops 10% NH_4HSO_3 soln or satd $NaHSO_3$ soln to clear soln and again boil to expel oxides of N. Remove from heat, cool to 20°, again add 0.5 g Na bismuthate, and stir. When max. permanagnate color develops, filter thru Alundum crucible or gooch contg asbestos mat that has been ignited, treated with 4% $KMnO_4$ soln, and washed with H_2O. Wash ppt with H_2SO_4 (1+9) until washings are colorless. Transfer filtrate to colorimeter tube. Compare color developed with stds similarly prepd by treating solns contg known quantities of the std $MnSO_4$ with the dil. HNO_3, NH_4HSO_3 or $NaHSO_3$ soln, and Na bismuthate. Color may also be compared with that of stds prepd from the $KMnO_4$ soln by dilg portions of 0.2, 0.4, 0.6 ml, etc., of the $KMnO_4$ soln with the dil. H_2SO_4 to same vol. as filtrate.

Iodide and Bromide—First Action

(This method is qualitative and approx. quant. For accurate quant. methods for iodides, *see* **31.064.**)

31.039 DETERMINATION

Evap. aliquot of alk. filtrate, **31.034,** to dryness; add 2–3 ml H_2O to dissolve residue and enough alcohol to make ca 90% alcohol. (This ppts chlorides.) Heat to boiling, filter, and repeat soln and pptn once or twice. Add 2 or 3 drops 10% NaOH soln to combined alc. filtrates and evap. to dryness. Dissolve last residue in 2–3 ml H_2O and repeat pptn with alcohol, heating, and filtering. Add drop of the NaOH soln to this alc. filtrate and evap. to dryness.

Dissolve residue in little H_2O; acidify with H_2SO_4 (1+5), using 3 or 4 drops excess; and transfer to small flask. Add 4 drops *0.2% $NaNO_2$ soln* and ca 5 ml CS_2. Shake until all I is extd and filter off acid soln from the CS_2. Wash flask, filter, and contents with cold H_2O and transfer the CS_2 contg the I in soln to Nessler tube, using ca 5 ml CS_2. In washing filter, make contents of tube to definite vol., usually 12–15 ml, and compare color with that of other tubes contg known quantities of I dissolved in CS_2. Prep. these std tubes by treating measured quantities of soln of known KI content as described above, beginning "acidify with H_2SO_4 (1+5) . . ."

Transfer separately to small flasks acid soln of sample and stds from which I has been removed. To stds add definite measured quantities of bromide soln of known concn, and to each flask contg sample and stds add 5 ml CS_2. Add *satd and freshly prepd Cl-H_2O*, 1 ml at time, shaking after each addn until all Br is set free. Avoid large excess of Cl, as a bromo-chloride may form and spoil color reaction.

Filter off aq. soln from the CS_2 thru moistened filter, wash contents of filter 2 or 3 times with

H_2O, and then transfer to Nessler tube with ca 1 ml CS_2. Repeat extn of filtrate twice, using 3 ml CS_2 each time. Combined CS_2 exts usually total 11.5–12 ml. Add enough CS_2 to tubes to bring them to definite vol., usually 12–15 ml, and compare sample with stds. If, when using this method near its upper limit, quantities of CS_2 recommended do not ext. all Br, make 1 or 2 addnl extns with CS_2; transfer exts to another tube; and compare color with some of lower stds. Add readings thus obtained to others.

Results closely approximating true values for I and Br can be obtained in shorter time on most samples by omitting extns with alcohol and comparing color of the CS_2 solns directly in extn flasks.

Bromide in Presence of Chloride but Not Iodide (*9*)—First Action

31.040 REAGENT

Alkaline sodium sulfite soln.—Dissolve 4 g Na_2SO_3 and 0.8 g Na_2CO_3 in H_2O and dil. to 100 ml.

31.041 APPARATUS

(a) *Reaction cylinder.*

(b) *Two high-form gas washing bottles.*

Join reacting cylinder and 2 gas washing bottles as in Fig. 62.

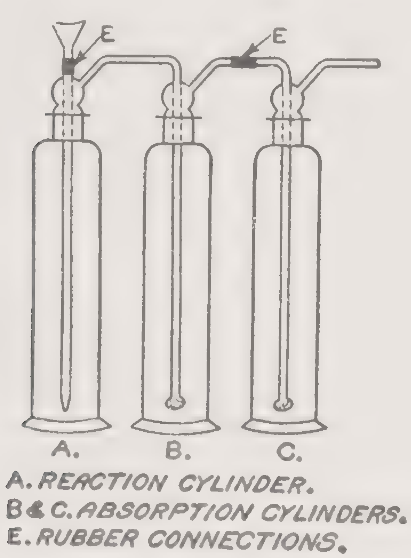

FIG. 62.—REACTION CYLINDER TO BE USED IN DETERMINATION OF BROMIDE

31.042 REACTIONS

$$2CrO_3 + 6HBr = Cr_2O_3 + 3H_2O + 3Br_2.$$
$$2H_2CrO_4 + 3H_2O_2 = Cr_2O_3 + 3O_2 + 5H_2O.$$
$$Na_2SO_3 + 2Br + H_2O = 2HBr + Na_2SO_4.$$

31.043 DETERMINATION

Take quantity of sample contg not >10 g total salts (sample should not be too acid). Evap. to dryness or nearly so.

Charge cylinder *A* by inserting glass beads to depth of ca 1″, followed by 15 g CrO_3 crystals and finally enough glass beads to fill cylinder half full. Add 20 ml of the alk. Na_2SO_3 soln to cylinder *B* and 5 ml to cylinder *C*. Dil. each to ca 200 ml. Connect 3 cylinders and draw current of air thru slowly.

Wash sample into cylinder *A* with enough H_2O to make ca 25 ml soln. Aspirate until contents of this cylinder are in soln and thoroly mixed, close inlet tube with small piece of rubber tubing and clamp, and reduce pressure in app. slightly by suction to guard against any possible escape of Br at ground-glass stopper. Let stand overnight and then aspirate with rather strong current of air (0.5–0.75 L/min.) 3 hr, adding four 2 ml portions 3% H_2O_2 soln to reaction flask at 30 min. intervals. Stop aspiration and evap. contents of cylinders *B* and *C* nearly to dryness.

Clean out cylinder *A* and freshly charge with glass beads and 15 g CrO_3 crystals. To cylinder *B* add 10 g KI dissolved in 200 ml H_2O and to *C*, 3 or 4 g KI in like quantity of H_2O. Connect app., draw thru slow current of air, and transfer contents of evapg dish to cylinder *A* thru small funnel, using 25 ml H_2O. Aspirate until all Br is evolved (ca 1 hr) and titr. the KI soln with std 0.05*N* $Na_2S_2O_3$. 1 ml $Na_2S_2O_3$ soln = 3.996 mg Br.

Bromide in Presence of Chloride and Iodide (*10*)—Official

(In presence of Cl and I only ca 95% of the Br present is recovered when 80 mg Br is contained in portion of sample taken for analysis. Method is satisfactory in absence of I.)

31.044 REAGENT AND APPARATUS—*See* **31.040** and **31.041**

31.045 REACTIONS

$$Fe_2(SO_4)_3 + 2KI = 2FeSO_4 + I_2 + K_2SO_4.$$
$$2CrO_3 + 6HBr = Cr_2O_3 + 3H_2O + 3Br_2.$$
$$2H_2CrO_4 + 3H_2O_2 = Cr_2O_3 + 3O_2 + 5H_2O.$$
$$Na_2SO_3 + 2Br + H_2O = 2HBr + Na_2SO_4.$$

31.046 DETERMINATION

Place 10 ml sample in distn flask, adjust vol. to ca 75 ml, and add 1.5–2.0 g $Fe_2(SO_4)_3.9H_2O$. Steam distill liberated I, discarding distillate. Transfer residue from distn flask to beaker, heat to boiling, add few drops Me orange, and ppt the Fe with NH_4OH, avoiding excess of NH_4OH, as a ppt of $Ca(OH)_2$ is bulky and difficult to wash. Filter off $Fe(OH)_3$, wash with hot H_2O, and evap. filtrate and washings to dryness or nearly so, taking care that during evapn soln does not become acid from hydrolysis of $MgCl_2$. Proceed as in **31.043**, beginning "Charge cylinder A . . . "

Arsenic—Official

31.047 REAGENTS AND APPARATUS—*See* **24.001** and **24.002**

31.048 DETERMINATION

Take portion of alk. filtrate, **31.034,** contg not >0.03 mg As_2O_3. If quantity taken is >10 ml, evap. soln to ca that vol. on steam bath. Transfer soln into generator of app. described in **24.002,** with aid of ca 10 ml H_2O, add 20 H_2SO_4 (1+2), and proceed as in **24.005,** beginning "add 5 ml of the KI reagent . . . "

Boric Acid

(Glassware contg B must not be used in these detns)

31.049 *Qualitative Test—Procedure*

Evap. part of alk. filtrate, **31.034,** to dryness, treat with 1–2 ml H_2O, and slightly acidify with HCl (1+1). Add ca 25 ml alcohol, boil, filter, and repeat extn of residue. Make filtrate slightly alk. with NaOH soln and evap. to dryness. Add little H_2O, slightly acidify with the dil. HCl, and place strip of turmeric paper in liquid. Evap. to dryness on steam bath and continue heating until turmeric paper is dry. If H_3BO_3 is present, turmeric paper turns cherry-red. As confirmatory test, apply drop NH_4OH (1+1) to reddened paper. Dark olive color is due to H_3BO_3.

Quantitative Method (11)—First Action

31.050 REAGENTS

(**a**) *Bromothymol blue indicator.*—1% alc. soln. (pH 6.0–7.6).

(**b**) *Sodium hydroxide std soln.*—0.0231N.

(**c**) *Boric acid soln.*—1 ml = 0.1 mg B.

(**d**) *Mannitol.*—Crystals. (Blank titrn for 5 g should not be >0.1 ml of the std NaOH soln.)

31.051 DETERMINATION

To 250 ml sample contg not >1 mg B in 600 ml beaker add 1 drop bromothymol blue indicator, acidify with 1N H_2SO_4, and add ca 0.5 ml excess. Bring to boil, stir cautiously, then boil vigorously 1 min. to expel CO_2. Cover beaker with watch glass and cool to room temp. in H_2O bath.

Place glass and calomel electrodes of pH meter and mechanical stirrer into beaker, start stirrer, and add CO_2-free 0.5N NaOH to approx. neutrality as indicated by the bromothymol blue. Adjust sample to pH 7.1 with ca 0.02N H_2SO_4 or the std NaOH soln if necessary. (Indicator needle of pH meter should be steady and should not drift from reading of 7.1.) Record microburet (5 ml, graduated in 0.02 ml) reading of the 0.0231N NaOH at this point. Add ca 5 g mannitol. (If B in form of H_3BO_3 is present, pH will drop to some value <7.1.) Titr. back to initial pH of 7.1 with the 0.0231N NaOH, using care near end point because of slight lag in the pH meter. With needle indicator remaining steadily on 7.1, record vol. of the NaOH soln used to nearest 0.01 ml. Subtract blank correction for reagents from total vol. 0.0231N NaOH used. (Substitute double-distd H_2O for sample, and carry out detn as above.)

Equivalency of the std NaOH soln in terms of B is obtained by titrg aliquots H_3BO_3 soln, (**c**). If the std base is exactly 0.0231N, 1 ml = 0.25 mg B, and if 250 ml sample is taken, each ml NaOH soln = 1 ppm B.

31.052 **Method of Reporting Results (*12*)—Procedure**

Report radicals and anhyd. salts in mg/L or, in case of highly concd waters, in g/L. For benefit of physicians, in case of medicinal waters, report also salts in terms of grains/quart, using factor 0.014600 to convert mg/L to grains/quart. In reporting salts in terms of grains/quart, convert salts that have H_2O of crystn to hydrated form as expressed in USP and in National Formulary, and convert $Mg(HCO_3)_2$ to $MgCO_3$ and $Ca(HCO_3)_2$ to $CaCO_3$. Use following factors in these calcns:

$$Na_2SO_4 \times 2.2682 = Na_2SO_4.10H_2O$$
$$MgSO_4 \times 2.0476 = MgSO_4.7H_2O$$
$$CaSO_4 \times 1.2647 = CaSO_4.2H_2O$$
$$Mg(HCO_3)_2 \times 0.5762 = MgCO_3$$
$$Ca(HCO_3)_2 \times 0.6174 = CaCO_3.$$

When complete analysis is made, report error of analysis and state how it is distributed. Report only significant figures.

Report Fe and Al together when present in unimportant quantities and in calcns consider them as Fe. When Fe and Al are present in larger quantities, make sepn and report each separately.

In calcg hypothetical combinations of acidic and basic ions, join NO_2, NO_3, BO_3, and AsO_4 to Na; I and Br to K; and PO_4 to Ca. Assign residual basic ions in following order: NH_4, Li, K, Na, Mg, Ca, Sr, Mn, Fe, and Al; to residual acid ions in following order: Cl, SO_4, CO_3, and HCO_3. In case not enough HCO_3 is present to join with all Ca, residual Ca is joined to SiO_2 to form $CaSiO_3$, and Mn, Fe, and Al are calcd to oxides Mn_3O_4, Fe_2O_3, and Al_2O_3, resp.

Use equiv. combining wts or their reciprocals in uniting radicals and, when necessary for purpose of comparison, in reducing salts to radicals and reuniting radicals in order specified above.

Equiv. combining wt of radical is obtained by dividing its wt by its valence. Equiv. combining wt of salt is obtained by dividing its molecular

wt by product of valency of basic element and number of atoms of basic element in the salt.

Procedure in calcg hypothetical combinations by use of equiv. combining wts and their reciprocals is as follows:

Multiply wts obtained, expressed in mg/L, or, for highly concd waters, in g/L, for each radical to be combined, by corresponding reciprocal of equiv. combining wts. If Na and K are to be detd by calcn, as is frequently the case, subtract sum of values obtained (reacting values) for basic radicals from sum of reacting values for acid radicals. Difference represents reacting value of undetd Na and K.

When all constituents in the water have been detd, sums of reacting values of acid and basic radicals should be very nearly same. In this case, if difference is reasonable and well within limit of accuracy of methods used, it may be distributed equally among all radicals detd, or among those that analyst believes to be less accurately detd than others. If difference is unreasonably great, repeat analysis in whole or in part. Sums of reacting values of acid and basic radicals must be equal before calcn is made. Obtain reacting values of the salts by subtracting in succession reacting values of radicals in specified order. To convert these values to mg/L of respective salts multiply each of them by the equiv. combining wt of respective salt.

31.053 *Equivalent Combining Weights and Their Reciprocals Based on International Atomic Weights, 1958*

Negative Radicals	Equivalent Combining Weights	Reciprocals of Equivalent Combining Weights	Positive Radicals	Equivalent Combining Weights	Reciprocals of Equivalent Combining Weights
NO_3	62.008	0.01613	NH_4	18.0404	0.05543
BO_2	42.82	0.02335	Li	6.940	0.14409
AsO_4	46.30	0.02160	K	39.100	0.02558
I	126.91	0.00788	Na	22.991	0.04348
Br	79.916	0.01251	Mg	12.16	0.08224
PO_4	31.658	0.03158	Ca	20.04	0.04990
HS	33.0681	0.03024	Sr	43.815	0.02282
S	16.03	0.06238	Ba	68.68	0.01456
SiO_3	38.03	0.02630	Mn	27.470	0.03640
O	8.0000	0.12500	Fe^{++}	27.92	0.03582
Cl	35.457	0.02820	Fe^{+++}	18.613	0.05372
SO_4	48.03	0.02082	Al	8.99	0.11123
CO_3	30.005	0.03333	Cu	31.785	0.03146
HCO_3	61.018	0.01639			

Salts	Equivalent Combining Weights	Reciprocals of Equivalent Combining Weights	Salts	Equivalent Combining Weights	Reciprocals of Equivalent Combining Weights
NH_4Cl	53.4974	0.01869	$MgSO_4$	60.19	0.01661
LiCl	42.397	0.02359	$MgCO_3$	42.16	0.02372
Li_2SO_4	54.970	0.01819	$Mg(HCO_3)_2$	73.159	0.01366
Li_2CO_3	36.945	0.02707	$Mg(NO_3)_2$	74.168	0.01348
$LiHCO_3$	67.959	0.01472	$CaCl_2$	55.497	0.01802
KCl	74.557	0.01341	$CaSO_4$	68.07	0.01469
K_2SO_4	87.130	0.01148	$CaCO_3$	50.045	0.01998
K_2CO_3	69.105	0.01447	$Ca(HCO_3)_2$	81.059	0.01234
$KHCO_3$	100.119	0.00999	$CaSiO_3$	58.07	0.01722
KI	166.010	0.00602	$Ca_3(PO_4)_2$	51.70	0.01934
KBr	119.016	0.00840	$SrSO_4$	91.845	0.01089
NaCl	58.458	0.01711	$SrCO_3$	73.82	0.01355
NaBr	102.907	0.00972	$Sr(HCO_3)_2$	104.834	0.00954
NaI	149.901	0.00667	$BaSO_4$	116.71	0.00857
Na_2SO_4	71.021	0.01408	$Ba(HCO_3)_2$	129.699	0.00771
Na_2CO_3	52.996	0.01887	$MnSO_4$	75.500	0.01324
$NaHCO_3$	84.010	0.01190	$MnCO_3$	57.465	0.01740
$NaNO_2$	68.999	0.01449	$Mn(HCO_3)_2$	88.490	0.01130
$NaNO_3$	84.999	0.01176	$FeSO_4$	75.95	0.01317
$NaBO_2$	65.811	0.01519	$Fe_2(SO_4)_3$	66.643	0.01500
Na_3AsO_4	69.294	0.01443	$FeCO_3$	57.925	0.01726
NaF	41.991	0.02381	$Fe(HCO_3)_2$	88.944	0.01124
NaHS	56.059	0.01784	Fe_2O_3	26.613	0.03758
Na_3PO_4	54.651	0.01829	$Al_2(SO_4)_3$	57.02	0.01754
Na_2S	39.021	0.02562	Al_2O_3	16.99	0.05886
Na_2SiO_3	61.021	0.01639			
$MgCl_2$	47.617	0.02100			

SALT (*13*)

31.054 Preparation of Sample—Procedure

If sample is coarser than "20 mesh," grind so that all will pass No. 20 sieve, but avoid undue grinding so that as much as possible will be retained on No. 80 sieve. Mix sample by quartering and weigh all needed portions as nearly at same time as possible.

31.055 Moisture—First Action

Place ca 10 g sample in dry, weighed 200 ml erlenmeyer. Weigh flask and sample. Spread sample evenly over bottom of flask by shaking gently and insert small funnel in neck. Heat flask and sample for periods of 1 hr each at ca 250° until 2 consecutive weighings agree within 5 mg. Shake flask occasionally so that sample will dry evenly. Report loss of wt as H_2O.

31.056 Matters Insoluble in Water—First Action

Place 10 g sample in 250 ml beaker, add 200 ml H_2O at room temp , and let stand 30 min., stirring frequently. Filter thru weighed gooch with asbestos mat dried at 110°. Transfer residue to gooch with aid of policeman, using total of not >50 ml H_2O. Wash residue with ca ten 10 ml portions H_2O, until 10 ml filtrate shows only faint opalescence upon addn of few drops $AgNO_3$ soln. Dry crucible and contents to constant wt at 110°. Report increase in wt gooch as "matters insol. in H_2O" and report results in % on H_2O-free basis. If matters insol. in H_2O are >0.1% det. their nature.

31.057 Matters Insoluble in Acid (*14*)—First Action

Treat 10 g sample with 200 ml HCl (1+19), boil 2–3 min., and let stand 30 min., stirring frequently. Filter thru gooch with mat dried at 110°. Wash, dry at 110°, cool, and weigh. Express results in %.

31.058 Preparation of Solution for Sulfate, Calcium, and Magnesium—Procedure

Weigh ca 20 g sample, transfer to 400 ml beaker, and dissolve in 200 ml HCl (1+3). Cover beaker, heat to boiling, and continue boiling gently 10 min. Filter thru paper and wash residue with small quantities of hot H_2O until filtrate is Cl-free. Unite filtrate and washings, cool, and dil. to vol. of 500 ml (Soln *A*).

31.059 Sulfate—First Action

Place 250 ml Soln *A*, **31.058**, in 400 ml beaker, heat to boiling, and add slight excess of hot 10% $BaCl_2$ soln dropwise while stirring. Conc. by heating gently and finally evap. to dryness on steam bath. Facilitate removal of free acid by stirring partly dried residue. Wash ppt by decantation with small quantities of hot H_2O, finally transferring ppt to close-grain filter paper with aid of policeman and stream of hot H_2O. Test filtrate for presence of Ba. Wash ppt on paper until filtrate is Cl-free. Dry and ignite paper contg ppt over Bunsen flame. Report % SO_4 in sample on H_2O-free basis.

31.060 Calcium—First Action

Place remainder of Soln *A* in 400 ml beaker. Add excess of 10% $H_2C_2O_4.2H_2O$ soln (10 ml usually is enough). Add few drops Me orange; neutralize while hot by adding NH_4OH dropwise, stirring constantly. Add ca 1 ml excess of the NH_4OH, stir, and let stand in warm place 3 hr. Decant supernatant thru filter, reserving filtrate for detn of Mg. Test filtrate for Ca with $(NH_4)_2C_2O_4$ soln. Wash ppt in beaker once with 10 ml 1% $(NH_4)_2C_2O_4$ soln, decanting thru filter paper. Combine filtrate and washings. Dissolve ppt on paper with hot HCl (1+1), using same beaker; dil. to 100 ml, add little more $H_2C_2O_4$ soln, and ppt as before. Let stand 3 hr, filter, and wash with the $(NH_4)_2C_2O_4$ soln as before, reserving filtrate and washings. Transfer ppt to crucible, dry, ignite, and heat over blast lamp to constant wt (CaO). Report as % Ca on H_2O-free basis.

31.061 Magnesium—First Action

Combine filtrates and washings from Ca detn, conc. if necessary by boiling gently to ca 150 ml, and proceed as in **31.026**. Report as % Mg on H_2O-free basis.

Iodine in Iodized Salt (*15*)—Official

31.062 REAGENTS

(a) *Bromine water.*—For alternative procedure, **31.064(b)**, det. approx. concn (mg Br/ml) by adding measured vol. from buret to flask contg 50 ml H_2O, 5 ml 10% KI soln, and 5 ml H_2SO_4 (1+9), and titrg liberated I with 0.1*N* $Na_2S_2O_3$.

(b) *Sodium thiosulfate.*—0.005*N*. Recently stdzd as in **42.036**.

(c) *Starch soln.* — 1% (freshly prepd). *See* **2.093(d)**.

(d) *Potassium iodide control soln.*—0.3280 g reagent-grade KI/250 ml. Dil. 50 ml to 250 ml, and use 5 ml (=1.0 mg I and 1.312 mg KI) for control.

31.063 PREPARATION OF SAMPLE

Dissolve 50 g sample in H_2O and dil. to 250 ml in vol. flask. Take 25, for **31.064(a)**, or 50 ml, for **31.064(b)**, aliquot for analysis.

31.064 DETERMINATION

(a) *Applicable when $Na_2S_2O_3$ content is not $>0.5\%$.*—Place sample aliquot in 600 ml beaker and dil. to ca 300 ml. Neutralize to Me orange with H_3PO_4 and add 1 ml excess. Proceed as in **22.071**, third paragraph.

(b) *Alternative procedure. Not applicable in presence of $Na_2S_2O_3$.*—Pipet 50 ml sample soln into 200 ml erlenmeyer. Neutralize to Me orange with $2N$ H_2SO_4. Add Br-H_2O dropwise from buret in quantity equiv. to 20 mg Br. After few min. destroy greater portion of remaining free Br by adding 1% Na_2SO_3 soln *dropwise while mixing.* Wash down neck and sides of flask with H_2O and complete removal of Br by adding 1 or 2 drops 5% phenol soln. Add 1 ml $2N$ H_2SO_4 and 5 ml 10% KI soln, and titr. liberated I with the $Na_2S_2O_3$ soln, adding 1 ml of the starch indicator near end of titrn. Correct detn for blank on reagents and make one or more control detns, using 50 ml 20% reagent-grade NaCl soln to which has been added appropriate quantities of the dil. control KI soln. 1 ml $0.005N$ $Na_2S_2O_3 = 0.1058$ mg I and 0.1388 mg KI.

31.065 Method of Reporting Results—Procedure

(In the absence of added drying agents such as $MgCO_3$, Ca phosphate, etc.)

Convert sulfate to $CaSO_4$ and unused Ca to $CaCl_2$, unless sulfate in sample exceeds quantity necessary to combine with Ca, in which case convert Ca to $CaSO_4$ and unused sulfate first to $MgSO_4$ and remaining sulfate, if any, to Na_2SO_4. Convert unused Mg to $MgCl_2$. Add percentages of $CaCl_2$ and $MgCl_2$. Report on H_2O-free basis % of matter insol. in H_2O, of SO_4, of Ca, of Mg, of $CaSO_4$, of $CaCl_2$, and of $MgCl_2$. Report also results of qual. examination of matters insol. in H_2O, if the quantity is $>0.1\%$ on H_2O-free basis.

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(*13*) Ibid. 384.

(*14*) Ibid. 385; **6**, 129(1923).

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32. Drugs

General Directions

32.001 EXTRACTION WITH LIGHTER-THAN-WATER SOLVENTS

Perform preliminary steps directed in method prior to extn. Ext. aq. soln in separator with specified vols of solvent (ether, petr. ether, etc.) by shaking not <1 min. Let sep. completely, swirl to remove H_2O droplets, transfer lower aq. layer to second separator, and decant solvent layer thru pledget of cotton in short-stem funnel inserted in neck of third separator. Wash mouth of separator with fine stream of solvent. Shake aq. soln repeatedly with addnl portions of solvent until substance sought is extd, using second and first separators alternately for shaking, collecting solvents by filtering into third. If aq. soln is to be further examined, dry cotton pledget in funnel by drawing air thru stem and wash with 5 ml H_2O into main aq. ext.

Sampling (*1*)—First Action

32.002 *I. Tablets and Pills*

(a) *Bulk lots.*—Mix lot as thoroly as possible without mutilating contents. Count, weigh, and powder thoroly at least 100 units. Calc. av. wt /unit.

(b) *Containers of 1000 or more units.*—Open and cautiously mix contents without mutilation. Count, weigh, and powder at least 30 units selected at random.

(c) *Containers of 100–500 units.*—Remove from one container at least 20 units selected at random, weigh, and powder.

(d) *Small containers, e.g., tubes of hypodermic tablets.*—Choose enough containers to provide at least 20 units, weigh, and powder contents.

(e) *Tablets or pills of small dosages, e.g., 1/100 grain of active ingredient.*—Number of units necessary may be so large as to make powdering unnecessary. Half or whole bottleful may be required. Count units to be used but do not powder.

II. Soft Capsules

Count and weigh at least 20 capsules and det. gross wt/capsule. Open capsules and transfer as much of contents as possible to weighing bottle. Clean capsules (cutting in 2 if necessary) and wash by agitating with alternate portions of alcohol and ether. (Few drops of HOAc mixed with the alcohol aids cleaning.) Remove ether before fan or air blast. Deduct wt cleaned, empty capsules from gross wt and calc. av. net contents.

III. Ampuls

Before opening ampuls dislodge any liquid in neck. Mark with file, or other suitable instrument, level of liquid on necks of at least 1 ampul if vol. is 10 ml or more; 3 or more if vol. is >3 ml and <10 ml; 5 or more if vol. is 3 ml or less. Open each near tip, transfer bulk of contents to small flask, and mix. To det. vol. contents, wash and dry empty ampuls, and fill to mark with H_2O from graduated pipet or buret.

ALCOHOLS

Acetone and Isopropyl and Ethyl Alcohols—Qualitative Tests (*2*)—Official

Acetone (In Absence of Other Ketones)

32.003 REAGENT

2,4-Dinitrophenylhydrazine soln.—Suspend 2 g 2,4-dinitrophenylhydrazine in 15 ml 2*N* HCl, add 10 ml HCl, then 600 ml 2*N* HCl, and filter.

32.004 TEST

To 1 ml sample add 5 ml of the reagent; acetone gives ppt. To obtain enough material for identification, treat 5 ml sample with 50 ml reagent. Swirl mixt. and let stand 15 min. Filter thru gooch and dry at 100°. Dissolve hydrazone in hot alcohol and filter; conc. filtrate to ca 5 ml; and let cool and crystallize. Acetone 2,4-dinitrophenylhydrazone melts at 128°.

Isopropyl and Ethyl Alcohols

32.005 REAGENTS

(a) *3,5-Dinitrobenzoyl chloride.*—Cryst. material from Eastman Kodak Co., m.p. 66–68°, can be used without further purification. If m.p. is $>70°$, indicating hydrolysis to 3,5-dinitrobenzoic acid, reconstitute to acid chloride as follows: Reflux 5 g of the material with 50 ml $SOCl_2$ until completely dissolved. Remove excess $SOCl_2$ by evapn on steam bath with air current. (CAUTION: Do not purify acid chloride by distn.)

(b) *Ether-petroleum ether solvent.*—Mix 1 vol. ether with 5 vols petr. ether. (3,5-Dinitrobenzoates are easily sol. in this solvent, whereas 3,5-dinitrobenzoic acid is practically insol.)

(**c**) *Lanthanum nitrate soln.*—Dissolve 0.5 g $La(NO_3)_3.6H_2O$ in 10 ml H_2O.

TEST

32.006 *I. When either alcohol is present in at least 5% aqueous solution*

Transfer 100 ml sample to 150 ml Claisen flask with long side arm bent downward at right angle ca 4″ from end. (Test tube immersed in ice-NaCl mixt. is convenient receiver.) Place anti-bumping stone in flask fitted with thermometer, and immerse flask in beaker of H_2O. Heat H_2O to boiling and continue distn until thermometer inside flask reaches 90°.

Add to receiver ca 1 g Na_2SO_4 and keep in refrigerator 1–2 hr to finish drying the alcohol. Filter thru small funnel into second test tube contg ca 0.1 g 3,5-dinitrobenzoyl chloride. Stopper tube immediately and immerse lower end in H_2O at 75–80°. Shake gently and continue heating 30 min. Cool soln, scratching side of tube to induce crystn. Ext. cryst. material with the ether-petr. ether solvent by filling tube with solvent and shaking. Filter into separator. Repeat extn 4 or 5 times. Ext. ether soln with 5% Na_2CO_3 soln, wash thoroly with H_2O, and filter thru funnel contg Na_2SO_4. Collect filtrate in beaker, evap. solvent, and det. m.p. of residue. Isopropyl 3,5-dinitrobenzoate melts at 122°, and ethyl 3,5-dinitrobenzoate at 92°.

32.007 *II. When either or both alcohols are present in approximately 1% aqueous solution*

Transfer ca 10 ml sample to 50 ml erlenmeyer and add 10 ml of the dinitrophenylhydrazine reagent, **32.003.** Formation of flocculent ppt of acetone 2,4-dinitrophenylhydrazone indicates presence of acetone. If acetone is present, proceed as in (**a**), and if absent, proceed as in (**b**).

(**a**) *Acetone present.*—Place 10 ml sample in 200 ml erlenmeyer and add 10 ml H_2O, 0.4–0.5 g *paraformaldehyde,* and 10 ml 5% NaOH soln. Heat to boiling under reflux. Continue heating until resin forms as indicated by appearance of ppt. While mixt. is still hot, add 50 ml *Fehling soln,* **29.035,** thru top of condenser. (Excess should be present, indicated by characteristic blue color.) Let mixt. cool, transfer to 500 ml Kjeldahl flask, and distill ca 50 ml into another Kjeldahl flask.

To distillate add 50 ml 10% $K_2Cr_2O_7$ soln, followed by 100 ml H_2SO_4 (1+3). Let mixt. stand 1 hr with occasional swirling; then add 100 ml 25% $FeSO_4.7H_2O$ soln and distill slowly. When 25 ml distillate collects, change receivers and collect addnl 50 ml.

Test first fraction with the dinitrophenylhydrazine reagent; ppt indicates presence of isopropyl alcohol. Test ca 5 ml second fraction for HOAc by adding 1 ml of the $La(NO_3)_3$ soln, followed by 1 ml 0.02*N* I and 1 drop NH_4OH. Heat over burner, and if HOAc is present (indicating Et alcohol), deep blue color develops.

(**b**) *Acetone absent.*—Transfer 10 ml sample to 50 ml erlenmeyer and add 10 ml $HgSO_4$ reagent, **9.037.** Place on steam bath and heat 15 min. White-yellow ppt indicates presence of isopropyl alcohol. If it is present, transfer 10 ml sample to Kjeldahl flask, add 50 ml H_2O, and proceed as in (**a**), beginning "add 50 ml 10% $K_2Cr_2O_7$ . . . "

If isopropyl alcohol is absent, test 10 ml sample by adding few drops 5% NaOH soln, excess of aq. I-KI soln, and warm. Odor of CHI_3 indicates presence of Et alcohol.

ALKALOIDS, OPIUM

32.008 Apomorphine in Tablets (*3*)—Official

Weigh number of tablets equiv. to ca 0.065 g (1 grain) of the alkaloid or its salt and dissolve in 10 ml H_2O in separator. Add 1 ml freshly prepd satd $NaHCO_3$ soln and 25 ml peroxide-free ether, and ext. as in **32.001,** using 15 ml portions ether for addnl extns. Discard aq. soln.

Wash ether soln of alkaloid 3 times with 5 ml portions H_2O, uniting aq. washings in clean separator. Ext. these washings with little fresh peroxide-free ether. Discard aq. portion, wash ether with H_2O, discard washings, and add washed ether to main portion of ether soln. Add 20.0 ml 0.02*N* H_2SO_4 to ether soln of alkaloid in separator and shake mixt. thoroly. Transfer both phases to beaker, wash separator twice with 5 ml portions H_2O, adding washings to beaker, and without delay evap. ether at low temp., preferably on H_2O bath with aid of air blast. Titr. excess acid with 0.02*N* NaOH, using 1 drop Me red, **32.023**(**b**). 1 ml 0.02*N* H_2SO_4 = 0.00625 g apomorphine hydrochloride, $C_{17}H_{17}O_2N.HCl.\frac{1}{2}H_2O$.

Codeine in Tablets (*4*)

32.009 *Qualitative Tests—Official*

(**a**) To residue or tablet add HNO_3. Solid turns orange, then yellow on soln.

(**b**) *See* Microchemical Tests, **32.224.**

32.010 *Quantitative Method—Official*

Transfer to small separator enough tablets, or powd. material equal to multiple of av. wt/tablet, contg ca 0.15 g of the alkaloid. Dissolve in min. quantity of H_2O (not >5 ml) acidified with 2 drops HCl. Add solid $NaHCO_3$ until neutralized, then slight excess, and ext. 5 times with $CHCl_3$, using 30, 20, 20, 10, and 5 ml. Test for complete

extn of alkaloid. (Make addnl extn with 10 ml $CHCl_3$, evap. solvent in sep. beaker, and dissolve residue in few drops MeOH. Add 1 drop Me red, **32.023**(**b**), and dil. with 20 ml CO_2-free H_2O. Yellow color indicates incomplete extn. Titr., and add quantity thus obtained to total.)

Combine $CHCl_3$ exts in second separator which has cotton pledget wet with $CHCl_3$ in stem. Wash combined exts with 1 ml H_2O contg 1 drop NH_4OH. When clear, filter into beaker. Ext. wash H_2O once with small portion $CHCl_3$. Evap. on H_2O bath, using elec. fan to prevent decrepitation of residue. When dry, remove immediately and complete detn by one of following methods:

(1) To alkaloidal residue add 2–3 ml MeOH, cover beaker with watch glass, and heat on steam bath until residue, including any portions that may adhere to upper part of beaker, completely dissolves. Add 2 drops Me red, **32.023**(**b**), and, without diln, titr. carefully with $0.02N$ H_2SO_4 to faint pink, avoiding excess. Cover beaker and digest on steam bath until all particles dissolve completely. If >2 ml MeOH is added, evap. excess. Cool, and dil. with 50 ml boiled H_2O (soln should now be yellow). Finish titrn with the std acid to faint red.

(2) Dissolve residue in 2–3 ml MeOH on steam bath. Add 2 drops of the Me red and then add from buret 5–10 ml excess of $0.02N$ H_2SO_4, noting total quantity used. Cover beaker with watch glass and heat on steam bath until residue, including any portions that may adhere to upper part of beaker, dissolves completely. Dil. with 50 ml cold, previously boiled H_2O. Back-titr. with the $0.02N$ NaOH. The H_2O and alkali should be sufficiently CO_3-free to insure sharp end point with Me red. 1 ml $0.02N$ $H_2SO_4 = 0.00787$ g codeine sulfate, $(C_{18}H_{21}O_3N)_2.H_2SO_4.5H_2O$, or 0.00849 g codeine phosphate, $C_{18}H_{21}O_3N.H_3PO_4.1\frac{1}{2}H_2O$.

Codeine and Terpin Hydrate in Elixirs (*5*)—First Action

32.011 REAGENTS

(**a**) *Color reagent.*—Either Folin-Denis reagent, **9.051**(a), or phosphotungstic-phosphomolybdic acid reagent prepd as follows: To 100 g pure Na tungstate and 20 g phosphomolybdic acid (free from nitrates and NH_4 salts), add 100 g H_3PO_4 and 700 ml H_2O. Boil over free flame 1.5–2 hr, cool, filter if necessary, and dil. to 1 L with H_2O. Equiv. quantity of pure molybdic acid may be substituted for phosphomolybdic acid.

(**b**) *Terpin hydrate std soln.*—Weigh accurately ca 80 mg terpin hydrate, add 2 ml HOAc, and stir until terpin hydrate is almost dissolved. Add 10 ml alcohol, stir, and transfer to 100 ml vol. flask. Rinse dish with three 10 ml portions alcohol. Finally rinse few times with H_2O and dil. to mark with H_2O. Soln keeps indefinitely.

(**c**) *Codeine std soln.*—Heat ca 100 mg codeine base 4 hr at 80° to obtain anhyd. codeine. Prep. soln contg 10 mg/100 ml $0.1N$ HCl.

32.012 DETERMINATION OF TERPIN HYDRATE

Pipet 5 ml sample into distg flask and add 100 ml satd NaCl soln, 35 ml alcohol, 2 ml HOAc, and 10 ml H_2O. Distill, collecting 100 ml distillate.

Pipet 5 ml color reagent into 50 ml vol. flask. Cool under running H_2O while slowly adding 5 ml H_2SO_4. Let mixt. come to room temp. and then add exactly 2 ml sample distillate. Place flask in boiling H_2O 20 min. Cool under H_2O to room temp. and dil. to mark with dil. alcohol (1+3). Shake every few min. until soln is clear (10–15 min.). (If soln fails to clear, phosphomolybdic acid used to prep. color reagent is unsatisfactory.)

Let stand 0.5 hr and det. absorbance at 725 mμ against reagent blank prepd without sample. Also det. absorbance of std soln prepd simultaneously with sample, beginning "Pipet 5 ml color reagent . . ." Terpin hydrate (g/100 ml elixir) $= A \times C \times 20/A'$; where A = sample absorbance, A' = std absorbance, and C = g terpin hydrate/100 ml std soln.

32.013 DETERMINATION OF CODEINE

Pipet 5 ml sample into separator, add 20 ml $0.1N$ HCl, and ext. with 50 ml ether. Drain aq. layer into 100 ml vol. flask. Wash ether once with 20 ml $0.1N$ HCl, drain acid into aq. layer, and dil. to mark with $0.1N$ HCl. Det. absorbance of this soln and of std soln at 285 mμ, against $0.1N$ HCl as reference. Codeine (g/100 ml elixir) $= A \times C \times 21.2/A'$, where A = sample absorbance, A' = std absorbance, and C = g codeine/100 ml std soln.

Diacetylmorphine (Heroin) in Tablets (*6*)

32.014 *Qualitative Test—Official*

See Microchemical Tests, **32.224**.

32.015 *Quantitative Method—Official*

Weigh, and transfer directly to small separator number of tablets contg ca 0.15 g diacetylmorphine. Dissolve in 5 ml H_2O contg 1 drop HOAc. Add 1 ml NH_4OH and ext. 5 times with $CHCl_3$, using 30, 20, 10, 10, and 5 ml, resp. Combine $CHCl_3$ exts in second separator which has cotton pledget wet with $CHCl_3$ in stem. Wash combined exts with 1 ml H_2O and proceed as in **32.010**, beginning "Evap. on H_2O bath . . ." 1 ml $0.02N$ $H_2SO_4 = 0.00847$ g diacetylmorphine hydrochloride, $C_{21}H_{23}O_5N.HCl.H_2O$.

32.016 **Dihydrocodeinone** (*7*)—**Official**

Weigh quantity of powd. sample contg ca 0.065 g dihydrocodeinone, transfer to separator, and

dissolve in 15 ml H_2O. Make alk. to litmus with dil. NH_4OH. Ext. with $CHCl_3$, using 30, 20, 20, and 10 ml portions. Filter into beaker and evap. combined $CHCl_3$ exts on H_2O bath to ca 5 ml, with aid of air current. Add 20.0 ml 0.02*N* H_2SO_4 and evap. remainder of the $CHCl_3$. Titr. excess acid with 0.02*N* NaOH, using Me red, **32.023(b)**. 1 ml 0.02*N* H_2SO_4 = 0.00707 g dihydrocodeinone hydrochloride, $C_{18}H_{21}O_3N.HCl.H_2O$.

32.017 Ethylmorphine in Sirups—Official

Pipet 50 ml sample into 250 ml separator, rinse pipet with H_2O, adding rinsings to separator, and dil. with H_2O to ca 100 ml. Make alk. with few drops dil. NH_4OH soln, and ext. with 50 ml portions $CHCl_3$-alcohol (9+1) (usually 4 extns are enough). Filter each ext. into same 250 ml beaker, and evap. combined exts on steam bath to ca 5 ml. Add 10.0 ml 0.02*N* H_2SO_4 and continue evapn until all $CHCl_3$ has evapd and alkaloids are in soln. Cool, add Me red, **32.023(b)**, and titr. excess acid with 0.02*N* NaOH. 1 ml 0.02*N* H_2SO_4 = 0.00772 g ethylmorphine hydrochloride, $C_{19}H_{23}O_3N.HCl.2H_2O$.

Heroin and Quinine—First Action

32.018 REAGENTS

(**a**) *Morphine hydrochloride.*—USP.

(**b**) *Sodium hydroxide-methanol soln.*—Dissolve 4 g NaOH in 200 ml MeOH and dil. to 1 L with H_2O.

32.019 PREPARATION OF STANDARD CURVE

Dissolve 92.6 mg morphine.$HCl.3H_2O$ in ca 20 ml anhyd. MeOH and dil. to 100 ml with 0.1*N* NaOH (concn = 1000 ppm as heroin.HCl). Dil. 10 ml of this soln to 100 ml with NaOH-MeOH soln. (1 ml = 100 mmg (100 ppm) as heroin.HCl.)

Dissolve 50 mg quinine.HCl in ca 20 ml anhyd. MeOH and dil. to 100 ml with 0.1*N* NaOH (concn = 500 ppm). Dil. 10 ml of this soln to 100 ml with NaOH-MeOH soln. (1 ml = 50 mmg (50 ppm) quinine.HCl.)

Det. absorbances (A_H) of std solns contg 0, 50, 100, 150 ppm heroin.HCl in NaOH-MeOH soln in matched silica cells on spectrophotometer at 297.5 and 330 mμ (A_H at 330 mμ should be 0); det. absorbances (A_Q) of std solns contg 0, 25, 50, 75 ppm quinine.HCl in NaOH-MeOH solns in matched silica cells at the same wavelengths. Plot the 3 std curves corrected for cell corrections $A_{H.297\cdot5}$, $A_{Q.297\cdot5}$, and $A_{Q.330}$, against concn in ppm, and calc. the corresponding absorptivities ($a = A$/concn, mg/L (ppm), in 1 cm cells).

32.020 DETERMINATION

Dissolve 100 mg sample in 10 ml anhyd. MeOH, and filter thru fritted glass funnel contg ¼" layer of H_2O- and MeOH-washed asbestos, using suction. Wash with several portions anhyd. MeOH to total vol. of ca 40 ml and combine alc. solns in 100 ml vol. flask. Dil. to mark with 0.1*N* NaOH. Dil. 10 ml aliquot to 100 ml with NaOH-MeOH soln. Det. absorbances of this soln at 297.5 ($A_{297\cdot5}$) and 330 mμ (A_{330}) in matched silica cells against NaOH-MeOH soln.

Calc. quinine.HCl concn in ppm (Q) from equation: $A_{330} = a_{Q.330} \times Q$. Calc. heroin.HCl concn in ppm (H) from equation: $A_{297\cdot5} = (a_{H.297\cdot5} \times H) + (a_{Q.297\cdot5} \times Q)$.

32.021 Morphine in Sirups (*8*)—**Official**

Shake bottle well and transfer 50 ml to 150 ml pear-shape separator. Add few drops NH_4OH (2+3) to insure weak alk. reaction and test with litmus paper. Ext. total alkaloids with $CHCl_3$-alcohol (9+1). (About seven 25 ml portions are necessary; number required depends on care used in sepg solvent and on length and violence of each shakeout. Larger quantities of solvent may be used to insure absence of emulsions.)

Combine solvents, wash with 5 ml H_2O, and filter solvent thru $CHCl_3$-wetted cotton. Evap. solvent. (If sirup is known to carry pure morphine alkaloid or its salt, and no other alkaloid, this residue may be dissolved in alcohol and filtered, and soln titrd.) Dissolve in 2 ml HCl (1+7) on H_2O bath, covering beaker to insure complete soln. Add 20 ml H_2O and transfer to separator. Make alk. with 5 ml 5% KOH soln and ext. with three 20 ml portions $CHCl_3$ followed by two 20 ml portions petr. ether to remove non-phenolic alkaloids and $CHCl_3$. Combine immiscible solvents and wash with 5 ml H_2O. Discard solvent and add wash H_2O to main aq. soln.

Acidify aq. soln with HCl (1+7) and then make just alk. with few drops NH_4OH (2+3), and ext. with three 20 ml portions petr. ether for removal of petr. ether-sol. phenolic alkaloids. Wash combined petr. ether ext. with 5 ml H_2O. Discard petr. ether and add wash H_2O to main aq. soln. Sat. with NaCl.

Ext. morphine completely with seven 25 ml portions $CHCl_3$-alcohol (9+1). Combine exts, wash with 5 ml H_2O, and filter thru plug of $CHCl_3$-satd cotton. Evap. to dryness and dissolve residue in 5 ml neutralized alcohol in covered beaker on steam bath. Add excess of 0.02*N* H_2SO_4 and back-titr. with 0.02*N* alkali, using Me red, **32.023(b)**. 1 ml 0.02*N* H_2SO_4 = 0.0076 g morphine sulfate, $(C_{17}H_{19}NO_3)_2.H_2SO_4.5H_2O$.

Morphine in Tablets (*6*)

32.022 *Qualitative Tests—Official*

(**a**) To residue or tablet add HNO_3. Orange-red color fading to yellow is produced.

(b) *See* Microchemical Tests, **32.224**.

Quantitative Method—Official

32.023 REAGENTS

(a) *Alkaline salt soln.*—Dissolve 30 g NaOH in H_2O, dil. to 1 L, add NaCl to satn, and filter.

(b) *Methyl red indicator.*—Dissolve 0.1 g Me red in 100 ml neutralized alcohol and filter if necessary.

32.024 PREPARATION OF SAMPLE

To det. variation in wt, weigh separately at least 20 tablets. Also weigh collectively representative number of unbroken tablets and calc. av. wt/tablet. To insure representative sampling in tablets contg $>\frac{1}{4}$ grain alkaloid, pulverize ca 20 tablets, mix powder thoroly, and protect from moisture in well-stoppered bottle.

32.025 DETERMINATION

Transfer to small separator enough tablets, or powd. material to contain ca 0.15 g of the alkaloid. Moisten with 5 ml H_2O, shake gently, and then dissolve completely by adding 10 ml of the alk. NaCl soln. (Excipients may not be completely sol.) To alk. NaCl soln add small piece litmus paper. Add HCl dropwise until neutral and then 10 drops excess. Add 5 ml alcohol, carefully neutralize with NH_4OH dropwise, and then add 5 drops excess. Invert separator and open stopcock to insure neutralization of residual acid. Immediately ext. with $CHCl_3$-alcohol (90+10) at least 6 times, using 30, 20, 20, 10, 10, and 5 ml, or until alkaloid is completely removed. Test for complete extn of alkaloid. (Make addnl extn with 10 ml $CHCl_3$-alcohol (9+1), evap. solvent in sep. beaker, dissolve residue in few drops MeOH, add 1 drop of the Me red, and dil. with 20 ml CO_2-free H_2O. Yellow color indicates incomplete extn. Titr., and add quantity thus obtained to total.)

Combine $CHCl_3$-alcohol exts in second separator which has cotton pledget wet with $CHCl_3$ in stem. Wash combined exts with 1 ml H_2O. When clear, filter into small beaker. Ext. wash H_2O twice with small portions of the $CHCl_3$-alcohol mixt. Evap. on H_2O bath, using elec. fan to prevent decrepitation of residue. When dry, remove immediately and complete detn by **32.010**(1) or (2). 1 ml $0.02N$ acid = 0.00751 g morphine hydrochloride, $C_{17}H_{19}O_3N.HCl.3H_2O$, or 0.0076 g morphine sulfate, $(C_{17}H_{19}O_3N)_2H_2SO_4.5H_2O$.

Alkaloids other than morphine are extd by $CHCl_3$, while morphine remains in the fixed alkali soln. In general this sepn is unnecessary. If tablets are of unknown composition or atropine or scopolamine is present, shake the alk. NaCl soln with 10 ml portions washed $CHCl_3$ (use ether for sepn of atropine). Transfer clear solvent to small beaker and evap. on steam bath. If residue is obtained, apply usual tests.

ALKALOIDS, OTHER THAN OPIUM

32.026 Aconitine in Aconite Root—Qualitative Test (*9*)—Procedure

Crush and macerate the aconite root in mortar with 15 ml H_2O and transfer to Mojonnier fat-extn tube. Add 5 ml NH_4OH (2+3) and ext. 2 or 3 times with 15 ml portions ether. Transfer ether ext. to separator and wash with H_2O. Ext. washed ether ext. with 2 or 3 ml $0.02N$ H_2SO_4. Test aq. layer with Me red, **32.023**(**b**); if alk., discard aq. layer. Continue to ext. with 2 or 3 ml $0.02N$ H_2SO_4 until aq. layer remains acid to Me red. Test slightly acid aq. layer for aconitine as follows:

In small test tube add 1 or 2 drops 5% Na_2CO_3 soln to 1 or 2 ml of the slightly acid aq. soln. Heat to 60°, stirring with thermometer. Cool and transfer few drops of the liquid to microscope slide and examine crystals. Aconitine forms irregular hexagonal plates. Most characteristic crystals of aconitine are formed in concn of 1/1000 or less.

Aminophylline and Phenobarbital (*10*)—Official

32.027 REAGENTS

(a) *Dilute ammonium hydroxide soln.*—0.1% NH_3. Dil. 4 ml NH_4OH to 1 L with H_2O.

(b) *Phenobarbital std soln.*—10 mmg/ml. Dissolve 100.0 mg phenobarbital in the dil. NH_4OH soln in 500 ml vol. flask, dil. to mark with the dil. NH_4OH, and mix. Transfer 5 ml aliquot to 100 ml vol. flask, dil. to mark with the dil. NH_4OH, and mix.

(c) *Theophylline std soln.*—10 mmg/ml. Dissolve 100.0 mg theophylline in HCl (1+18) in 500 ml vol. flask. Dil. to mark with the dil. HCl and mix. Transfer 5 ml aliquot to 100 ml vol. flask, dil. to mark with H_2O, and mix.

32.028 SEPARATION OF AMINOPHYLLINE AND PHENOBARBITAL

Transfer weighed portion of powd. sample contg ca 15 mg phenobarbital to separator contg 25 ml HCl (1+1). Add 60 ml ether, shake, and let stand to clear. Pass aq. soln successively thru 2 other separators, each contg 50 ml ether, shake, and let stand to clear. Transfer ether-washed aq. soln to 500 ml vol. flask. Wash the 3 ether solns successively with three 10 ml portions HCl (1+1) and one 10 ml portion H_2O, and add these washes to the vol. flask. Reserve for detn of theophylline.

32.029 DETERMINATION OF PHENOBARBITAL

Combine ether solns and evap. to dryness. Dissolve residue in ca 100 ml of the dil. NH_4OH and

transfer to 200 ml vol. flask. Dil. to mark with the dil. NH_4OH and mix. Filter, if necessary, transfer 10 ml aliquot to 100 ml vol. flask, dil. to mark with the dil. NH_4OH, and mix. Det. absorbance, A_P, at 240.5 mμ against solvent blank. Read this soln same day it is prepd.

Det. absorbance, A_P', of the std phenobarbital soln, **32.027(b)**, at 240.5 mμ, using the dil. NH_4OH as blank. Calc. absorptivity, $a_P = A_P'/cb$, where $c = 0.01$ g/L, and b = cell length in cm. Phenobarbital (g/L sample soln) $= A_P/a_P$.

If stearates are present, proceed as above, dissolving residue in ca 100 ml of the dil. NH_4OH and dilg to ca 190 ml with the NH_4OH. Acidify with HCl, testing with litmus paper. Dil. to vol. with H_2O, mix, and filter. Transfer 10 ml aliquot to 100 ml vol. flask, add 1 drop NH_4OH (1+1), dil. to vol. with the dil. NH_4OH, and det. absorbance, A_P, at 240.5 mμ.

32.030 DETERMINATION OF THEOPHYLLINE

Dil. aq. soln in vol. flask to mark with H_2O and mix. Transfer 5 ml aliquot, or aliquot contg 0.5–1.0 mg theophylline, to 100 ml vol. flask, dil. to mark with H_2O, and mix. Det. absorbance, A_T, at 271 mμ against blank soln contg same quantity HCl. Det. absorbance, A_T', of the std theophylline soln, **32.027(c)**, and calc. absorptivity, a_T, as in **32.029**. Theophylline (g/L sample soln) $= A_T/a_T$. Aminophylline = 1.33 × theophylline.

32.031 Arecoline Hydrobromide (*11*)—**Official**

Accurately weigh enough sample to insure 0.1–0.15 g arecoline hydrobromide. Rinse sample into 125 ml separator which has fairly tight-fitting cotton pledget moistened with $CHCl_3$ in stem. Add enough H_2O to separator to make total of 20 ml (enough to dissolve arecoline hydrobromide); to soln add solid $NaHCO_3$ until small quantity remains undissolved.

Ext. with $CHCl_3$, using 30, 25, 20, 15, and 10 ml portions, and test for complete extn. Drain ext. into 250 ml tall beaker or (preferably) 500 ml erlenmeyer. To beaker or flask add 35.0 ml 0.02*N* H_2SO_4, and evap. $CHCl_3$ on steam bath, using glass beads or stirring rod to prevent superheating. When $CHCl_3$ has evapd, cool acid soln and titr. with 0.02*N* NaOH, using 2 drops Me red, **32.023(b)**. 1 ml 0.02*N* H_2SO_4 = 0.00472 g $C_8H_{13}O_2N.HBr$.

Atropine in Tablets

32.032 *Extraction Method (12)—Official*

Count and weigh enough tablets to yield ca 0.065 g alkaloid and add directly to small separator. Dissolve in 5–20 ml H_2O and add 1 ml NH_4OH. Add equal vol. $CHCl_3$, shake, and let stand until sepn is complete. Drain $CHCl_3$ layer into second separator, repeat extn with fresh portions of solvent until alkaloid is completely removed, and combine all fractions. Wash combined $CHCl_3$ solns by shaking with 5 ml H_2O and let clear.

Insert pledget of absorbent cotton in stem of separator and drain $CHCl_3$ soln into small beaker. Add 10 ml $CHCl_3$ and shake. When H_2O seps completely, drain $CHCl_3$ into beaker. Wash outer surface of separator stem with little $CHCl_3$, adding washings to beaker. Evap. soln on steam bath to ca 5 ml. Add measured excess of 0.02*N* H_2SO_4 and continue evapn until odor of $CHCl_3$ disappears. Cool soln and back-titr. with 0.02*N* NaOH, using 1 drop Me red, **32.023(b)**. 1 ml 0.02*N* H_2SO_4 = 0.00578 g atropine or 0.00695 g atropine sulfate.

Infrared Method (137)—First Action

32.033 APPARATUS

Infrared spectrophotometer for operation in 2–15 μ region, equipped with 2 NaCl cells 1.0 mm thick, suitable for CS_2 solns. Absorbances of cells when filled with CS_2 should match to within 0.05. Use cell having higher absorbance for sample soln.

32.034 DETERMINATION

Transfer enough tablets to yield 5–10 mg atropine to small separator. Dissolve tablets in 5 ml H_2O, add 1 ml NH_4OH and 20 ml $CHCl_3$, and shake 1 min. Let sep. and filter $CHCl_3$ layer thru pledget of cotton moistened with $CHCl_3$ into 50 ml g-s. flask. Repeat extn with three 10 ml portions $CHCl_3$. Evap. combined $CHCl_3$ exts to dryness on steam bath with aid of current of air.

Transfer ca 25 mg atropine USP, accurately weighed, to 50 ml g-s. flask. By pipet, add to sample and std flasks measured vols of CS_2 sufficient to produce concns of ca 3 mg/ml. Stopper flasks, mix well, and immediately det. baseline absorbances, A_B, of sample and std solns relative to CS_2 at max. of 9.68 μ, drawing baseline between minima at 9.14 and 9.87 μ. Calc. atropine sulfate content of sample; atropine × 1.20 = atropine sulfate. Record spectra of sample and std solns from 2 to 15 μ and compare for identity of sample.

Belladonna and Stramonium Alkaloids in Ointments (*13*)—**Official**

32.035 *Method I.*

Weigh ca 25 g well-mixed sample into 250 ml separator contg loosely packed cotton pledget in stem; add 100 ml ether-$CHCl_3$ mixt. (4+1) and shake vigorously until all fats are dissolved. Ext. alkaloids with five 20 ml portions dil. H_2SO_4 (2%

is satisfactory), let settle, and drain clear aq. soln into smaller separator contg 10 ml ether. Wash each acid ext. successively thru this same 10 ml ether and drain acid solns into another 250 ml separator.

Make combined acidified solns alk. with NH_4OH and ext. alkaloids completely by shaking with five 25 ml portions $CHCl_3$. Each time let settle; then filter $CHCl_3$ thru cotton wetted with $CHCl_3$ into 250 ml beaker, finally washing stem of separator and filter with little $CHCl_3$. Evap. solvent carefully on H_2O bath with moderate heat to ca 10 ml; add measured excess (ca 10 ml) of $0.02N$ H_2SO_4, stir mixt., and continue evapn until all $CHCl_3$ is expelled. Add 20 ml recently boiled, cooled H_2O and 1 drop Me red, **32.023(b)**, and titr. excess acid with $0.02N$ NaOH. 1 ml $0.02N$ $H_2SO_4 = 0.00579$ g alkaloids of belladonna or stramonium leaves.

NOTE. It is recommended that the ointment be transferred by means of soft metal ointment tube or empty tooth paste tube, and weighed by difference. Assay can be hastened by centrifuging where instructions are given to let mixt. stand until it settles.

32.036 *Method II.*

Weigh ca 25 g sample into tall beaker. Add ca 5 g *paraffin*, 25 ml 2% H_2SO_4, and 10 ml ether. Warm mixt. gently on steam bath until fluid, stirring thoroly. Continue this procedure until most of ether has evapd. Place beaker in ice bath until cold. Make several holes in paraffin layer with stirring rod and filter acid soln thru cotton pledget into small separator. Wash cake once with small quantity of H_2O, filtering washings thru cotton into separator. Wash acid with 10 ml ether and drain into 250 ml separator. Repeat treatment with 4 successive portions of acid and ether, filtering each portion thru the cotton into small separator and washing each ext. with same 10 ml portion ether. Combine acidified exts, make alk. with NH_4OH (2+3), and ext. and titr. alkaloids as in **32.035**.

Butacaine Sulfate (*14*)—Official

32.037 REAGENTS

(**a**) *Potassium iodide soln.*—20%. Prep. fresh.

(**b**) *Starch indicator.*—Make 1.5 g sol. starch into paste with few ml H_2O, and add slowly, with stirring, to 300 ml boiling H_2O.

(**c**) *Picrolonic acid soln.*—2.5% in alcohol.

32.038 DETERMINATION

(**a**) *Ointments containing butacaine sulfate in petrolatum or other greasy base.*—Accurately weigh into 125 ml separator sample contg ca 50 mg butacaine sulfate. Add 25 ml benzene and swirl until ointment base dissolves; then add 10 ml HCl (1+7) and shake separator gently ca 1 min. Let layers sep., drain aq. phase into second separator, and repeat extn 4 times with 10 ml portions H_2O. Wash combined aq. exts with 5 ml CCl_4 and discard washing. Neutralize soln with NH_4OH, add 2 ml excess, and ext. butacaine base by shaking with five 15 ml portions $CHCl_3$. Filter each ext. thru cotton pledget into 100 ml beaker, and evap. combined exts on steam bath in current of air until no $CHCl_3$ odor remains.

Rinse down wall of beaker with 2 ml alcohol delivered from pipet, warm until oily base dissolves completely, and add 1 drop HCl. Tilt and rotate beaker to wet with acidic soln any liquid on wall of beaker, and add 1 drop Me red, **32.023(b)**. If soln does not react strongly acid, add enough HCl dropwise. Dil. with few ml H_2O, and quantitatively wash into 500 ml I flask with more H_2O.

To soln add, from pipet, 10 ml KBr-$KBrO_3$ soln, **32.128**, dil. to 200 ml with H_2O, and add 10 ml HCl. Immediately stopper flask and swirl 5 min. or until ppt coagulates. After 5 min. add 5 ml of the KI soln to flask, stopper, and shake vigorously. Rinse stopper and neck of flask with little H_2O, and titr. soln with $0.1N$ $Na_2S_2O_3$, **42.035**, until color is discharged. Add 15 ml of the starch indicator and 20 ml $CHCl_3$, stopper flask, and shake vigorously. Continue titrn, vigorously shaking stoppered flask after each addn of $Na_2S_2O_3$ soln. Add $Na_2S_2O_3$ soln dropwise as end point approaches. (During titrn, mixt. passes thru series of color changes; at end point aq. phase is colorless and emulsified $CHCl_3$ layer is nearly so.) 1 ml $0.1N$ $Na_2S_2O_3 = 0.00889$ g $(C_{18}H_{30}N_2O_2)_2.H_2SO_4$.

To isolate bromination product for identification, transfer titrd mixt. to separator, make alk. with NH_4OH, and shake vigorously. Drain emulsified $CHCl_3$ layer, and to break emulsion, filter with suction thru 0.5 cm layer of Hiflo Super-Cel (or similar filter-aid) supported on paper in büchner. Shake aq. phase remaining in separator with 25 ml $CHCl_3$, and pass $CHCl_3$ ext. thru filter. Transfer combined filtrates to separator, filter $CHCl_3$ layer thru cotton pledget into beaker, and evap. on steam bath in air current.

To oily residue of dibromobutacaine add 2 ml of the picrolonic acid soln and stir. Filter ppt on Hirsch funnel, wash with 2–3 ml alcohol, dry at 105°, and det. capillary m.p., alone and in admixture with authentic dibromobutacaine picrolonate (m.p. 158–160° with decomposition). If ppt does not form on adding the picrolonic acid soln to bromination product, seed with small crystal of dibromobutacaine picrolonate; if ppt still does not form, butacaine is absent.

(**b**) *Tablets.*—Accurately weigh 20 tablets and det. av. wt/tablet. To 125 ml separator add enough accurately weighed, finely powd. tablet

mixt. to provide ca 200 mg butacaine sulfate, add 25 ml H_2O, and swirl separator until sample dissolves. Add 2 ml NH_4OH and ext. with six 15 ml portions $CHCl_3$. Shake each ext. with 5 ml H_2O in second separator, and then filter thru cotton pledget into beaker. (If emulsion forms in aq. phase in first separator, more than 6 extns may be required. Test for complete extn by evapg seventh ext. on steam bath; if appreciable residue is obtained, dissolve it in $CHCl_3$, combine with previous exts, and continue extns until complete. If aq. phase in first separator tends to emulsify, break emulsion by addn of Na_2SO_4 or by other means.) Evap. filtrate to small vol. on steam bath and complete detn by one of following methods:

(1) Transfer the concd soln of butacaine base quantitatively to tared 50 ml beaker with $CHCl_3$, remove solvent by heating on steam bath in air current, dry 30 min. at 105°, cool in desiccator and weigh. Wt residue × 1.160 = wt butacaine sulfate, $(C_{18}H_{30}N_2O_2)_2.H_2SO_4$.

Gravimetric detn may be checked acidimetrically as follows: Rinse down wall of beaker with 2 ml neutral alcohol delivered from pipet, warm beaker on steam bath until butacaine base dissolves completely, add 1 drop Me red, **32.023**(**b**), and rinse down beaker wall with another 2 ml alcohol. Titr. soln with $0.1N$ H_2SO_4, **42.038** or **42.039**, almost to point of color change; rinse down wall of beaker with H_2O, dil. to ca 45 ml, and complete titrn. 1 ml $0.1N$ H_2SO_4 = 0.0356 g $(C_{18}H_{30}N_2O_2)_2.H_2SO_4$.

(2) Det. gravimetrically as in (1); then proceed as in (**a**), beginning "Rinse down wall of beaker with 2 ml alcohol . . . " except to use 50 ml instead of 10 ml of the KBr-$KBrO_3$ soln.

(3) Completely remove solvent on steam bath, and proceed as in (1), second paragraph. Then wash titrd soln into 500 ml I flask, pipet in 50 ml KBr-$KBrO_3$ soln, **32.128**, dil. to 200 ml with H_2O, add 10 ml HCl, and proceed as in (**a**), beginning "Immediately stopper flask . . . "

(**c**) *Crystals.*—Accurately weigh ca 200 mg sample into 125 ml separator, add 25 ml H_2O, and swirl separator until sample dissolves. Continue as in (**b**), beginning "Add 2 ml NH_4OH. . ."

(**d**) *Solutions.*—Transfer to 125 ml separator aliquot contg ca 200 mg butacaine sulfate, and if necessary dil. to 25 ml with H_2O. Proceed as in (**b**), completing detn by (**b**)(1), (2), or (3) if chlorobutanol is absent, and only by (**b**)(3) if chlorobutanol is present.

Cocaine (*15*)—Official

32.039 *Method I.*

Weigh accurately uniformly mixed sample contg 0.1–0.2 g alkaloid. Transfer to small separator and dissolve in min. quantity of H_2O. Make soln slightly alk. with NH_4OH and ext. with successive small portions of peroxide-free ether until alkaloid is completely removed from aq. soln, using mercuric-KI reagent, **28.025**(**j**), for test. Combine ether exts, evap. most of ether on steam bath, and let remainder evap. spontaneously at room temp. Dissolve residue in few ml neutral alcohol, add 20.0 ml $0.05N$ H_2SO_4, and titr. excess of acid with $0.02N$ NaOH, using Me red, **32.023**(**b**). 1 ml $0.05N$ H_2SO_4 = 0.01699 g cocaine hydrochloride, $C_{17}H_{21}O_4N.HCl$.

32.040 *Method II.*

Weigh accurately uniformly mixed sample contg ca 0.2 g alkaloid. Dissolve in 20 ml cold H_2O, add 2 drops HCl (1+3), and transfer to separator. Make alk. to litmus with freshly prepd satd $NaHCO_3$ soln, and ext. completely with petr. ether (four 20 ml portions are usually enough). Filter combined exts thru plug of absorbent cotton into separator, and wash cotton with petr. ether. Add decided excess of $0.02N$ H_2SO_4, accurately measured, and shake vigorously several min. Sep. 2 layers and wash petr. ether with two 10 ml portions H_2O, adding washings to acid soln. Titr. excess acid with $0.02N$ alkali, using the Me red, **32.023**(**b**), and reserve titrd soln for check detn described below. 1 ml $0.02N$ H_2SO_4 = 0.006793 g cocaine hydrochloride, $C_{17}H_{21}O_4N.HCl$.

As check, add 10 ml $2.5N$ NaOH to titrd alkaloidal soln and evap. on steam bath to ca 10 ml. Cool, transfer soln to separator, and acidify with HCl (1+3). Ext. completely with successive portions of $CHCl_3$. Filter combined exts thru plug of absorbent cotton and wash cotton well with $CHCl_3$. Let $CHCl_3$ soln evap. spontaneously in weighed beaker, dry residue 2 hr in vac. desiccator, and weigh. From wt benzoic acid found calc. its equiv. of cocaine hydrochloride. $C_6H_5COOH \times 2.782 = C_{17}H_{21}O_4N.HCl$. (Benzoic acid may also be detd by titrn.)

Demerol (Meperidine, Pethidine) (*16*)—Official

Distillation Method

32.041 APPARATUS

App. consists of 500 ml round-bottom, short-neck flask to which is fitted adapter with attached separator (Ace Glass Co. No. 5270). Connect distg head (Scientific Glass App. Co. No. J-1148) fitting this adapter to straight inner-tube, H_2O-cooled condenser by another adapter (Ace No. 5125). Third adapter attached to bottom of condenser dips below surface of liquid in 1 L erlenmeyer. All joints are ₸ 24/40.

32.042 PREPARATION OF SAMPLE

Weigh not <20 tablets and reduce to fine powder without appreciable loss.

32.043 DETERMINATION

Weigh accurately powder contg ca 100 mg demerol and wash into 500 ml round-bottom flask with ca 25 ml H_2O. Add ca 1 g powd. $CaCO_3$ to flask and connect to distn app. Place 1 L erlenmeyer contg 20 ml 0.02*N* H_2SO_4 under condenser so that adapter on end of condenser is below surface of acid. Add 100 ml H_2O to flask thru separator, and distill until ca 25 ml remains in flask. Without interrupting distn, add second 100 ml H_2O slowly enough so that distillate does not suck back into distn flask. Continue distn until this portion has distd over. In like manner distill over third 100 ml H_2O. Then add 10 ml alcohol thru separator. When most of alcohol distills over, add and distill over 10 ml portion H_2O.

Disconnect condenser from distn app. and rinse inside of condenser and adapter that dipped into the std acid, catching rinsings in receiving flask. Bring collected distillate to vigorous boil to remove any dissolved CO_2, cool, and titr. excess acid with 0.02*N* NaOH, using Me red, **32.023**(**b**). 1 ml 0.02*N* H_2SO_4 = 0.005673 g demerol hydrochloride, $C_{15}H_{21}O_2N.HCl$.

32.044 *Extraction Method*

Weigh accurately portion of powder, prepd as in **32.042**, contg ca 0.1 g demerol, and macerate 2 hr with 10 ml H_2O and 1 ml 1*N* H_2SO_4. Decant liquid thru small filter into separator. Macerate residue 20 min. with 5 ml H_2O, filter thru same filter, and wash residue and filter with small portions of H_2O.

Sat. soln with NaCl; then add 5 ml 1*N* NaOH and ext. with 25 ml and six 20 ml portions ether as in **32.001**. Wash combined ether exts with two 5 ml portions H_2O; then ext. this H_2O with 10 ml ether and add this ether to main ether ext. Ext. ether soln first with 20.0 ml 0.02*N* H_2SO_4, and then successively with 10 and 5 ml H_2O. Combine H_2SO_4 and H_2O exts in beaker and warm on H_2O bath until no ether odor is detected. Cool soln, and titr. excess acid with 0.02*N* NaOH, using Me red, **32.023**(**b**). 1 ml 0.02*N* H_2SO_4 = 0.005673 g demerol hydrochloride, $C_{15}H_{21}O_2N.HCl$.

32.045 **Emetine Hydrochloride in Tablets** (*17*)—**Official**

Transfer to small separator powd. sample, **32.002**, accurately weighed, contg ca 0.1 g alkaloidal salt. Dissolve in min. quantity of H_2O and add 5 ml 4% NaOH soln. Ext. as in **32.001** with 30 ml washed ether and with 25, 20, 15, and 10 ml portions ether or until extn is complete, washing each ext. with sep. 1 ml portion H_2O, adding wash H_2O to aq. soln. Filter combined ether exts into beaker thru cotton previously wet with ether; finally wash separator with ether, evap. bulk of ether on steam bath, and complete evapn at low temp.

To residue add 2 ml neutral alcohol, cover beaker with watch glass, and let reflux on steam bath few min. Add few drops of Me red, **32.023**(**b**), and, without dilg, titr. with 0.02*N* acid to faint pink. Cover beaker and digest on steam bath until all particles dissolve completely. Cool, and add ca 30 ml recently boiled H_2O. Finish titrn with std acid to faint red. 1 ml 0.02*N* H_2SO_4 = 0.00553 g emetine hydrochloride, $C_{29}H_{40}O_4N_2.2HCl$.

32.046 **Ephedra, Alkaloids in** (*18*)—**Official**

Place 10 g ephedra, in fine powder, in erlenmeyer. Add exactly 100 ml ether-$CHCl_3$ mixt. (3+1) (cooled to working temp. after mixing). Stopper securely, shake, and let stand at least 5 min. Add 5 ml NH_4OH (2+3) and 0.5 g anhyd. Na_2CO_3, stopper tightly, and macerate at least 4 hr, with occasional shaking. Decant or filter rapidly 50 ml aliquot clear supernatant (representing 5 g drug), transfer to separator, and shake with 2% H_2SO_4, using 15, 10, 10 ml portions, etc., until extn is complete.

Combine acid solns in separator, neutralize with the NH_4OH, and add ca 5 g anhyd. Na_2CO_3, stirring until dissolved. Ext. with ether as in **32.001**, using 35, 30, 25, 20, and 15 ml portions, or until extn is complete. Filter combined ether exts into small beaker thru cotton pledget previously wet with ether.

Evap. ether to ca 10 ml on steam bath with moderate heat. Add bromothymol blue, **4.111**(**e**), measured excess of 0.02*N* H_2SO_4, and ca 40 ml CO_2-free H_2O. Cover with watch glass, return to steam bath to dissolve any alkaloid on sides of beaker, and then evap. ether. Titr. excess acid with 0.02*N* alkali. 1 ml 0.02*N* acid = 0.00330 g ephedra alkaloids calcd as ephedrine.

Ephedrine in Inhalants—Official

32.047 *Method I.* (*19*)

Weigh accurately 5–10 g sample into small tared beaker. Add 10 ml 2% H_2SO_4, stir, and let stand ca 15 min. Transfer to small separator, rinsing beaker with small portions of ether. Shake gently, and transfer acid layer to second separator. Shake with three 10 ml portions 2% H_2SO_4, rinsing beaker with ether each time. Test for complete removal of alkaloid.

Neutralize combined acid solns in separator with NH_4OH and add 5 ml excess. Ext. soln with 30 ml washed ether (automatic extractor optional), transfer aq. layer to second separator, and wash ether ext. with 1 ml H_2O, adding washings to main aq. soln. Swirl ether to remove H_2O adhering to side of separator. After all H_2O has

been removed, filter mixt. into erlenmeyer thru cotton pledget wet with ether inserted in small funnel. Repeat extn with liberal portions of washed ether at least 4 times, or until alkaloid is removed completely, washing each portion with same 1 ml H_2O. Evap. ether to 10 ml in air current. Add bromothymol blue, **4.111(e)**, measured excess of 0.02*N* H_2SO_4, and ca 40 ml CO_2-free H_2O; cover with watch glass, heat on steam bath to dissolve alkaloid on sides of flask, and evap. all ether. Cool, and titr. excess acid with 0.02*N* NaOH, using indicator std, pH 6.0, **13.024,** for comparison. 1 ml 0.02*N* H_2SO_4 = 0.00330 g ephedrine.

32.048 *Method II.* (*20*)

(**a**) *Oily inhalants containing oxazolidines* (*products of reaction of ephedrine with carbonyl compounds*).—Accurately weigh or otherwise measure sample contg ca 100 mg ephedrine. (With most inhalants, sample size will be ca 10 ml. Altho sample contg as little as 20 mg of the drug may be used if necessary, larger sample should be employed when practicable.) Rinse sample quantitatively into 125 ml erlenmeyer with portions of reagent-grade benzene totaling ca 5 ml, add 10 ml 5% H_2SO_4, and boil gently 10 min. with frequent agitation and swirling. (Boiling is best done on hot plate and should be conducted carefully to avoid superheating, bumping, and loss of sample.)

Cool flask, transfer contents to 125 ml separator, and rinse erlenmeyer with portions of benzene totaling ca 1.5 times vol. sample in order to remove all oily matter from flask. (Preferably use lipped flask to facilitate quant. transfer of contents to separator.) Shake separator contg the acid and benzene rinsings, drain acid layer into second separator, and swirl first separator vigorously to force down addnl acid and insure more nearly complete sepn of phases. Drain into second separator any acid layer that further seps, and ext. benzene-oil phase with the three 5 ml portions H_2O previously used to rinse flask. Swirl first separator each time after main portion of H_2O has been drained into second separator. (In transfer of ephedrine from org. solvent to aq. phase, and *vice versa*, shake at least 1 min. and as vigorously as possible without causing troublesome emulsions.)

Wash acid soln of ephedrine sulfate with 3 ml $CHCl_3$ and discard $CHCl_3$ washings. Make soln alk. to litmus with 20% NaOH soln (ca 2.5 ml); then add 0.5 ml excess and ext. the ephedrine by shaking *vigorously* with six 15 ml portions $CHCl_3$. If >50 mg ephedrine is present, filter exts thru cotton pledget into tared 100 ml beaker previously dried at 110° and cooled in desiccator. After fourth extn, rinse filter funnel and its tip with $CHCl_3$ (letting rinsings drain into beaker), float 5 drops (0.2 ml) HCl onto surface of combined exts, and evap. on steam bath in air current until beaker can easily accommodate remaining exts. To test for complete extn, shake alk. phase with seventh and eighth 15 ml portions $CHCl_3$, filter these thru cotton into small beaker, float 2 drops HCl onto surface, and evap. to dryness on steam bath in air current. If *cryst.* residue results, combine it with main exts, using little MeOH for transfer, and repeat test if considered necessary.

Continue evapn of main exts to 1 or 2 ml. Then cautiously heat on bath, but without air current, until no HCl odor remains and residue of ephedrine hydrochloride is apparently dry. Heat beaker 30 min. at 110°, cool in desiccator, and weigh. Wt residue × 0.8192 = wt ephedrine base.

If <50 mg ephedrine is present in sample, carry out extn to completion as described previously, and evap. filtered exts in untared beaker until $CHCl_3$ (but not excess HCl) has been removed. Direct fine stream of redistd MeOH around inside of beaker to dissolve ephedrine salt, and immediately repeat process with stream of $CHCl_3$. Transfer MeOH-$CHCl_3$ soln to tared 20 ml beaker, previously dried at 110° and cooled in desiccator, and repeat MeOH and $CHCl_3$ rinsings until ephedrine hydrochloride has been quantitatively transferred. Evap. soln on steam bath, in air current, until the salt begins to crystallize. Continue removal of solvent by cautious heating alone, to avoid loss from decrepitation, until residue is apparently dry and there is no odor of HCl. Dry and weigh as previously directed.

(**b**) *Oily inhalants or petroleum jelly preparations containing free ephedrine only.*—If product is oil, quantitatively transfer suitable sample, (a), to 125 ml separator with portions of benzene totaling ca 1.5 times vol. sample, and ext. mixt. with 5 ml H_2SO_4 (1+17) and then with four 5 ml portions H_2O, swirling separator each time as in (**a**), and continue assay as in (**a**), beginning "Wash acid soln of ephedrine sulfate . . . " If product is petr. jelly prepn, dissolve sample in enough benzene to obtain soln of suitable fluidity (30 ml should be enough for 10 g sample) and proceed as for oily inhalants.

32.049 Ephedrine in Water-Soluble Jellies, Sirups, and Solutions of Ephedrine Salts—Official

(**a**) *Water-soluble jellies.*—If product is thin jelly of viscosity similar to that of the N. F. X prepn, accurately weigh ca 10 g sample. If it is thick jelly, reduce size of sample (which should contain 20–100 mg of the alkaloid) to ca 5 g to diminish possibility of emulsion formation during ether extns. Transfer quantitatively to separator

with aid of H_2O, and dil. with H_2O until vol. jelly and H_2O is ca 20 ml. Make mixt. slightly alk. to litmus with 20% NaOH soln, add addnl 0.5 ml, and quantitatively ext. ephedrine with ether, using 3-separator technic as in **32.001.** In each extn use vol. ether equal to that of aq. phase, and swirl separator vigorously after draining bulk of aq. layer to obtain efficient sepn of 2 phases. If equilibrium is attained in each extn 5 or 6 will be enough. Ext. ether with 5 ml 10% H_2SO_4 and four 5 ml portions H_2O as in **32.048**(**b**), and continue assay as in **32.048**(**a**), beginning "Wash acid soln of ephedrine sulfate . . ."

(**b**) *Sirups.*—Use 10 ml sample, measured by vol. flask, and proceed as in (**a**).

(**c**) *Solutions of ephedrine salts.*—Use accurately measured sample contg ca 100 mg ephedrine and proceed as in (**a**), but if sample is <20 ml, diln to this vol. before extg is unnecessary.

Ephedrine in Tablets and Capsules —Official

32.050 *Method I.* (*21*)

Weigh accurately quantity of powd. sample contg ca 0.12 g of the alkaloidal salt, and transfer to separator. Dissolve in min. quantity of H_2O; then add 5 ml NH_4OH. Ext. soln with 30 ml washed ether. Transfer aq. layer to second separator. Wash ether ext. with 1 ml H_2O, adding washings to main aq. soln. Swirl ether to remove H_2O on side of separator. After all H_2O has been removed, filter into beaker thru cotton pledget wet with ether inserted in small funnel. Repeat extn with liberal portions of ether at least 4 times, or until alkaloid is completely removed, washing each portion with 1 ml H_2O. Evap. ether to ca 10 ml with air current and proceed as in **32.047,** beginning "Add bromothymol blue . . ." 1 ml $0.02N$ acid = 0.00403 g ephedrine hydrochloride, 0.00428 g ephedrine sulfate, and 0.00330 g ephedrine.

32.051 *Method II.* (*20*)

Weigh accurately sample of capsule contents or finely powd. tablets (preferably equiv. to ca 100 mg drug). Transfer to separator contg 20 ml H_2O and make alk. with ca 0.5 g anhyd. Na_2CO_3. Continue as in **32.049**(**a**), beginning with ether extns, but make the acid ext. alk. with anhyd. Na_2CO_3 instead of 20% NaOH soln. Use ca 0.5 g excess of that required to make soln alk. to litmus.

Ergotamine in Tablets (*22*)—First Action

(Applicable in presence of caffeine, acetophenetidin, phenobarbital, and belladonna alkaloids)

32.052 REAGENTS

(**a**) *Tartaric acid soln.*—1%. Dissolve 10 g tartaric acid in H_2O and dil. to 1 L.

(**b**) *Alcoholic tartaric acid soln.*—Mix equal vols tartaric acid soln, (**a**), with alcohol. Prep. fresh daily.

(**c**) *Sodium bicarbonate soln.*—10%. Dissolve 100 g $NaHCO_3$ in H_2O and dil. to 1 L.

(**d**) *Citric acid soln.*—(1+1). Mix equal wts of citric acid and H_2O.

(**e**) *Alum soln.*—$0.025M$. Dissolve 12 g $KAl(SO_4)_2.12H_2O$ in H_2O and dil. to 1 L. pH should be 3.5 ± 0.2.

(**f**) *Color reagent.*—Dissolve 1.25 g *p*-dimethylaminobenzaldehyde in cooled mixt. of 650 ml H_2SO_4 and 350 ml H_2O. Add 0.5 ml 9% $FeCl_3$ soln.

(**g**) *Diatomaceous silica support.*—Celite 545. Boil 150 g with 1 L HCl (1+1) 10 min. and cool. Wash with H_2O to remove acid and dry in oven at ca 100°. Dried product should give negative test for acid when moistened.

(**h**) *Ergotamine tartrate std soln.*—50 mmg/ml. Dissolve 25 mg ergotamine tartrate, USP, in enough tartaric acid soln, (**a**), to make 500 ml.

32.053 PREPARATION OF CHROMATOGRAPHIC COLUMN

Chromatographic tube.—Prep. chromatographic tube from 25×200 mm test tube. Fit with packing rod ca 400 mm long with flat head 22–23 mm diam. Place small wad of glass wool in bottom of tube.

Ergotamine-retaining layer.—Add ca 4 g Celite to 3 ml citric acid soln in beaker. Mix thoroly with scoop-shaped spatula until mixt. appears fluffy and uniform, and transfer to chromatographic tube. Tap side of tube gently to settle mixt. Press down firmly with packing rod.

Ergonovine-retaining layer.—Add ca 2 g Celite to 2 ml alum soln, mix, and transfer to tube on top of citric acid layer. Press down firmly and evenly.

Water layer.—Add ca 2 g Celite to 2 ml H_2O, mix, and transfer to tube on top of alum layer. Press down firmly and evenly.

32.054 PREPARATION OF SAMPLE

Weigh counted number of not <10 tablets and reduce to fine powder. Weigh portions equiv. to 2.5 mg ergotamine tartrate into beaker. Mix thoroly with 5 ml 1% tartaric acid soln and let stand 30 min. Add 5 ml $CHCl_3$ and 1 ml 10% $NaHCO_3$ soln and mix. (Aq. phase must be alk.) Add ca 7 g Celite and stir thoroly until mass appears uniform and does not stick to beaker. It may be necessary to wash down sides of beaker with small amounts of $CHCl_3$. Add and mix more Celite as may be necessary to make mixt. workable. Transfer mixt. quantitatively to another chromatographic tube fitted with glass wool plug, in several portions, pressing down firmly with packing rod. Wash packing rod, spatula,

and sides of beaker with small amount (ca 5 ml) $CHCl_3$. Add enough Celite to make mixt. workable. Scrub sides of beaker and add mixt. to tube. Again rinse rod, spatula, and beaker with $CHCl_3$ and pour wash onto column.

32.055 SEPARATION OF ERGOTAMINE

Place tube contg sample so that effluent will flow directly onto water layer of second column. Add 50 ml H_2O-satd ether to top column and receive eluate from bottom column in 250 ml erlenmeyer. Follow with 50 ml H_2O-satd $CHCl_3$. (Since effluent may flow faster thru sample column than thru second column, do not add too much $CHCl_3$ at a time.) Rinse tip of sample column with $CHCl_3$ from wash bottle and discard sample column.

Let column drain completely and then rinse down sides with small amount $CHCl_3$. Pass thru addnl 25 ml H_2O-satd $CHCl_3$ into same flask and rinse tip of column with alcohol. Discard effluent if ergotamine was properly retained. (*See* Note.)

Inspect the column for proper retention of ergotamine and for presence of H_2O-sol. alkaloids by holding column under ultraviolet light *very briefly*. Blue fluorescent band must not be at bottom of column. (*See* Note.) Extrude the column into 400 ml beaker. Add 8 g $NaHCO_3$ and ca 25 ml H_2O to form aq. liquid layer. Break up column with spatula and mix. Wash mixt. with H_2O from wash bottle into 250 ml separator. Add 10 ml $CHCl_3$ and shake. Check aq. layer to assure that it is alk. Drain $CHCl_3$ layer thru glass wool filter into 100 ml vol. flask. Ext. aq. layer with four 10 ml portions $CHCl_3$ and filter solvent layers into the 100 ml vol. flask. Dil. to vol. and mix.

32.056 DETERMINATION

Evap. 10 ml aliquot of $CHCl_3$ soln in 50 ml erlenmeyer to dryness with air current. Do not heat. (Ergotamine is easily decomposed. If assay cannot be completed in 1 day, dried residue after evapn of $CHCl_3$ may be stored in refrigerator overnight.) Dissolve residue, equiv. to 0.25 mg ergotamine tartrate, in 5.0 ml alc. tartrate soln. Pipet 5.0 ml std soln into 50 ml erlenmeyer. Add to each, 10 ml color reagent while swirling continuously in ice-H_2O bath. After 30, but <60 min., det. absorbances at 550 mμ of sample, A, and of std, A', relative to blank prepd by mixing 5 ml H_2O and 10 ml reagent.

Mg ergotamine tartrate in sample weighed $=(A/A')\times 2.5$.

Note: Ergot alkaloids fluoresce bright blue when exposed to ultraviolet light at ca 360 mμ. If fluorescent band has reached bottom of trap layer, sample must be discarded. If desired, sample can be salvaged by combining column and effluent, shaking with $CHCl_3$, and passing thru another acid trap. Use of eluent which is not H_2O-satd will cause loss of ergotamine from column. Blue fluorescent ring at top of alum layer indicates presence of H_2O-sol. ergot alkaloids. If detn of H_2O-sol. alkaloid content is desired, repeat detn on new portion of sample, changing citric acid trap to one prepd by mixing 3 g Celite with 3 ml alum soln. Cover alum layer with mixt. of 2 g Celite and 2 ml H_2O.

32.057 Homatropine in Tablets (*23*)—Official

Weigh accurately quantity of sample, prepd as in **32.002,** contg ca 0.130 g alkaloidal salt, and transfer to separator. Dissolve in 10–20 ml H_2O and add 2 ml NH_4OH. Add ca 20 ml $CHCl_3$, shake, and let stand until sepn is complete. Drain $CHCl_3$ layer into second separator and repeat extn with fresh portions of solvent until alkaloid is completely removed (5 extns usually suffice). Test for complete removal with I soln, **32.098(b)**. Combine all fractions and wash $CHCl_3$ soln with 5 ml H_2O.

Filter $CHCl_3$ soln thru cotton into small beaker. Wash aq. soln with 10 ml $CHCl_3$; drain $CHCl_3$ and filter into beaker. Wash outer surface of stem of separator, funnel, and funnel stem with little $CHCl_3$, adding washings to beaker. Evap. soln on steam bath to ca 5 ml. Add measured excess of $0.02N$ H_2SO_4. Place beaker in warm place and evap. with aid of fan until no $CHCl_3$ odor remains. Cool soln and back-titr. with $0.02N$ NaOH, using 1 drop Me red, **32.023(b)**. 1 ml $0.02N$ $H_2SO_4=0.00713$ g homatropine hydrobromide or 0.00626 g homatropine hydrochloride.

Ipecac Alkaloids in Fluidextract (*24*)—Official

32.058 PREPARATION OF SOLUTION

Pipet 20 ml sample into 100 ml vol. flask, add ca 5 ml $1N$ H_2SO_4, and with aid of air blast evap. on steam bath to ca 10 ml. Then, while rotating flask, add ca 30 ml H_2O, cool to room temp., and dil. to mark with H_2O. Let stand overnight and filter thru dry filter, rejecting first few ml filtrate.

DETERMINATION

32.059 *Automatic Extraction Method*

Measure 20 ml prepd filtrate (4 ml fluidextract of ipecac) into automatic extractor, Fig. 63, B, fitted to 200 ml erlenmeyer. Add 60 ml H_2O, 2 ml NH_4OH (1+5), and ca 50 ml peroxide-free ether. Shake gently to prevent deposition of any solid matter on bottom of extractor and then add peroxide-free ether until ca 75 ml passes over into flask. Heat flask on steam bath (not elec. hot plate) and ext. 2 hr, or until extn is complete.

Sep. ether from aq. layer and add it to main concentrate in flask. Evap. combined ether exts on steam bath, add 2–3 ml absolute alcohol, and repeat evapn to remove all traces of NH_3. Warm alkaloidal residue on steam bath with 2–3 ml

neutral alcohol to insure complete soln. Add 10 ml 0.1N H_2SO_4, and dil. with ca 20 ml recently boiled, cooled H_2O. Titr. excess of acid with 0.02N NaOH, using Me red, **32.023(b)**. 1 ml 0.1N H_2SO_4=0.0240 g ether-sol. alkaloids of ipecac.

32.060 *Hand Extraction Method*

(More rapid than automatic extraction method and yields results almost as high)

Pipet 20 ml prepd filtrate, **32.058**, into separator. Add 2 ml NH_4OH (1+5), and ext. as in **32.001** with 20 ml portions peroxide-free ether until extn is complete (at least 8 times), using Mayer reagent, **28.025(j)**, as test. Wash combined ether exts with ca 10 ml H_2O, and then wash this wash H_2O with little peroxide-free ether, adding ether washings to main soln. Transfer ether soln to 200 ml erlenmeyer, and evap. ether on steam bath with aid of air blast. Add 2–3 ml absolute alcohol and repeat evapn to remove all traces of NH_3. Warm alkaloidal residue with 2–3 ml neutral alcohol to insure complete soln, and titr. as in **32.059**.

32.061 Physostigmine in Ointments (*25*)—Official

Weigh accurately 5 g well-mixed ointment directly into 125 ml erlenmeyer and insert small glass rod for mixing and transfer purposes. Add 10 ml H_2SO_4 (1+85), melt ointment completely on steam bath, and mix contents thoroly by swirling flask. Cool in pan of cracked ice, swirling contents occasionally until solidified. With aid of glass rod, filter acid soln into 250 ml separator thru cotton pledget inserted in stem of funnel. Repeat extn 4 more times, and wash funnel and cotton pledget with few ml H_2O. Continue as in **32.062**, line 4, beginning "Make alk. to litmus with solid $NaHCO_3$ . . . "

32.062 Physostigmine Salicylate in Tablets (*26*)—Official

Weigh accurately enough powd. sample, prepd as in **32.002**, to contain ca 0.065 g physostigmine salicylate, transfer to separator, and add enough H_2O (not >20 ml) to dissolve. Make alk. to litmus with solid $NaHCO_3$, and ext. at once with $CHCl_3$, using 30, 20, 10, and 10 ml portions. Transfer each ext. to second separator contg 5 ml H_2O. Wash each ext. with this H_2O and filter solvent into beaker, using cotton pledget moistened with $CHCl_3$. Test for complete extn by making addnl extn with 5 ml portion $CHCl_3$ and treating separately as below. Evap. combined $CHCl_3$ exts on H_2O bath to ca 5 ml in air current, remove beaker from bath, and complete evapn without heat.

Dissolve residue in few ml neutral alcohol. Add

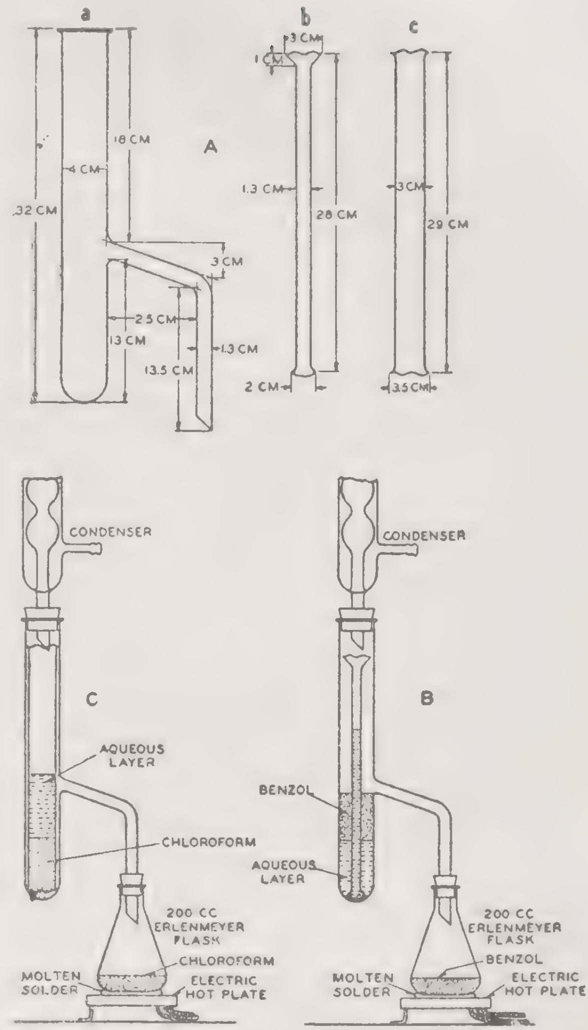

FIG. 63.—CONTINUOUS EXTRACTION APPARATUS

excess of 0.02N H_2SO_4. Cover with watch glass and heat on steam bath until alkaloids are washed down sides by refluxing action. Remove watch glass and evap. bulk of alcohol. Cool, add Me red, **32.023(b)**, and titr. excess acid with 0.02N NaOH. 1 ml 0.02N H_2SO_4=0.00827 g physostigmine salicylate, $C_{15}H_{21}O_2N_3.C_7H_6O_3$.

32.063 Pilocarpine Hydrochloride in Tablets (*27*)—Official

Det. av. wt/tablet. Pulverize, mix thoroly, and weigh sample contg ca 0.065 g of the salt. Dissolve sample in 10 ml H_2O, add 1 ml NH_4OH (2+3), and shake out rapidly with 20 ml $CHCl_3$. Repeat extn, using 15 ml $CHCl_3$, and complete extn with successive 10 ml portions. Filter each portion of $CHCl_3$ thru cotton pledget and combine in 250 ml beaker, finally washing stem of separator and funnel with $CHCl_3$. Evap. $CHCl_3$ on steam bath to ca 5 ml. Add 20.0 ml 0.02N H_2SO_4 and evap. remainder of $CHCl_3$. Titr. excess acid with 0.02N NaOH, using 1 drop Me red,

32.023(b). (End point is not sharp, but with care it can be obtained.) 1 ml 0.02*N* H_2SO_4 = 0.00489 g pilocarpine hydrochloride, $C_{11}H_{16}O_2N_2.HCl$.

32.064 Plasmochin (Pamaquin) (*28*)—**Official**

Weigh powd. sample equiv. to ca 0.2 g plasmochin naphthoate and transfer to 100 ml beaker. Add 10 ml H_2O and 5 ml HCl (1+3), and stir until powder is thoroly wet. Add 20 ml H_2O and stir again. Filter thru Selas crucible or gooch, and wash with several portions of H_2O until filtrate is colorless.

Transfer filtrate completely to separator, make alk. with NH_4OH, and ext. with several portions of $CHCl_3$. (Test for complete extn can be made by evapg sep. ext. to dryness and adding 1*N* acid; yellow color should not be produced. If ext. is yellow, it can be returned to separator and reextd.) Wash $CHCl_3$ exts with several ml H_2O, filter thru cotton, evap. to ca 5 ml, and then add 25 ml 0.02*N* acid. Continue heating, and when bulk of $CHCl_3$ evaps, stir with glass rod to facilitate removal of last droplets of $CHCl_3$, avoiding prolonged heating of soln. Cool, and titr. to purple tint with 0.02*N* alkali, using bromocresol purple, **32.103.** 1 ml 0.02*N* acid = 0.01406 g plasmochin naphthoate, or 0.0063 g plasmochin base.

Procaine—Official

32.065 QUALITATIVE TESTS

See Microchemical Tests, **32.224.**

QUANTITATIVE METHODS

32.066 *Method I.*

(Dets as procaine any *p*-aminobenzoic acid formed by decomposition)

Dissolve quantity of sample equiv. to ca 0.1 g procaine hydrochloride in 5 ml H_2O in 50 ml beaker. Add 25 ml 0.1*N* NaOH and heat on steam bath 25 min. Cool and transfer soln to 500 ml g-s. flask. Add 50 ml std KBr-$KBrO_3$ soln, **32.128,** dil. with H_2O to 250 ml, add 10 ml HCl, and stopper flask immediately to avoid loss of Br. Shake flask occasionally and let stand 2 hr at room temp., keeping flask tightly stoppered. (It is necessary that large excess of Br, as shown by bright yellow color, be present.) Add quickly 10 ml 20% KI soln, stopper, and shake flask. Let stand 15 min., shaking occasionally. Titr. excess I with 0.1*N* $Na_2S_2O_3$, using starch indicator, **4.004(f)**. Titr. to disappearance of blue color, disregarding color that reappears on standing. 1 ml 0.1*N* KBr-$KBrO_3$ = 0.00454 g procaine hydrochloride, $C_{13}H_{20}O_2N_2.HCl$.

32.067 *Method II.*

(Dets only undecomposed procaine)

Weigh quantity of powder or number of tablets equiv. to ca 0.2 g procaine. Dissolve in 10–15 ml H_2O, transfer soln to separator, and add ca 3 ml NH_4OH. Ext. ammoniacal soln 4 or 5 times with $CHCl_3$, using 15 ml for first extn and 10 ml for subsequent extns. Filter into beaker and evap. $CHCl_3$ with elec. fan, preferably at room temp., avoiding prolonged heating of procaine base which is slightly volatile at 100°. Take up residue with slight excess of 0.1*N* or 0.02*N* H_2SO_4. Titr. excess acid with 0.02*N* NaOH, using Me red, **32.023(b)**. 1 ml 0.1*N* H_2SO_4 = 0.0273 g $C_{13}H_{20}O_2N_2.HCl$. 1 ml 0.02*N* H_2SO_4 = 0.00546 g $C_{13}H_{20}O_2N_2.HCl$.

32.068 *Method III.* (*29*)

(Applicable in presence of chlorobutanol, cocaine, codeine, heroin, lactose, and morphine)

Weigh into Kjeldahl flask 0.3–0.5 g procaine or its salts, or measure equiv. quantity of ampul soln. Dissolve in 150 ml H_2O (or add enough H_2O to make 150 ml), and add 2 ml 50% NaOH soln. Quickly connect to condenser and distill 100 ml into flask contg measured excess of std acid, extending delivery tube below surface of soln. Remove receiver, rinse condenser with little H_2O, and titr. excess acid with std alkali, using Me red, **32.023(b)**. 1 ml 0.1*N* acid = 0.0236 g procaine, $C_{13}H_{20}O_2N_2$, or 0.0273 g procaine hydrochloride, $C_{13}H_{20}O_2N_2.HCl$.

32.069 Prostigmine (Neostigmine) (*30*)—**Official**

Weigh portion of powd. sample equiv. to ca 0.06 g prostigmine bromide into 500 ml Kjeldahl flask. Add 200 ml H_2O, 25 ml NaOH (1+1), few crystals of $Ba(OH)_2$ to prevent foaming, and several glass beads. Distill dimethylamine formed by alk. hydrolysis into 25.0 ml 0.02*N* H_2SO_4, collecting at least 150 ml distillate. Titr. excess H_2SO_4 with 0.02*N* NaOH, using Me red, **32.023(b)**. 1 ml 0.02*N* H_2SO_4 = 0.00606 g prostigmine bromide.

Quinacrine Hydrochloride (Atabrine) (*31*)—**Official**

32.070 REAGENTS

(a) *Sodium acetate-acetic acid mixture.*—Dissolve 2.5 g $NaOAc.3H_2O$ and 1 ml HOAc in enough H_2O to make 45 ml.

(b) *Dilute hydrochloric acid.*—Dil. 15 ml HCl to 80 ml with H_2O.

(c) *Potassium iodide soln.*—Dissolve 16.5 g KI in H_2O and dil. to 100 ml.

32.071 DETERMINATION

Transfer accurately weighed sample equiv. to ca 0.25 g quinacrine hydrochloride to 100 ml vol. flask with aid of 45 ml of the NaOAc-HOAc mixt. Shake thoroly to dissolve the quinacrine hydrochloride. Let settle and add 50.0 ml 0.1*N* $K_2Cr_2O_7$. Dil. to mark with H_2O and mix well. Let stand

10–15 min. and filter thru dry paper, rejecting first 15 ml filtrate. Measure 50.0 ml filtrate into g-s. flask. Add 80 ml of the dil. HCl and 20 ml of the KI soln. Stopper flask and mix by gentle swirling. Let stand 5 min. and titr. liberated I with 0.1N $Na_2S_2O_3$, adding starch indicator as end point nears. 1 ml 0.1N $K_2Cr_2O_7$ = 0.00848 g $C_{23}H_{30}OClN_3.2HCl.2H_2O$.

Quinine—Official

Spectrophotometric Method (32)

32.072 REAGENT

Quinine std soln.—Recrystallize quinine N.F. twice from benzene and dry to constant wt at 100°. Prep. soln contg 5.0 mg of this anhyd. quinine in 100 ml 0.1N HCl. (Keeps indefinitely.)

32.073 DETERMINATION

(**a**) *In presence of compounds which have no absorption in HCl soln at 347.5 mμ.* (These compounds include strychnine, atropine, ephedrine, caffeine, acetylsalicylic acid, acetanilid, acetophenetidin, camphor, phenolphthalein, glycerol, alcohols; most green, blue, and red dyes; and sugars; all of which are frequently found in prepns contg quinine.)—Accurately weigh or measure sample contg ca 0.1 g quinine and transfer to 1 L vol. flask. Dissolve in 25 ml HCl (1+2) and dil. to vol. so that final concn is ca 0.1N in HCl. Filter soln if not perfectly clear. Pipet aliquot contg 2–5 mg quinine into 100 ml vol. flask and dil. to mark with 0.1N HCl. Det. absorbance, A, relative to blank of 0.1N HCl at 347.5 mμ, and absorbance, A', of the std soln relative to same blank.

Mg quinine (anhyd.) in aliquot = $5.0A/A'$.

(**b**) *Applicable in presence of ferric compounds, such as elixir of iron, quinine, and strychnine.*—Proceed as in (a), but add 10 ml H_3PO_4 to the soln in 100 ml flask before dilg to vol. Std soln should contain 10 ml H_3PO_4/100 ml, and blanks should consist of 0.1N HCl contg 10 ml H_3PO_4/100 ml.

(**c**) *Applicable in presence of interfering substances.*—Before detg quinine by its absorption at 347.5 mμ, sep. it from following compounds: Aloin, podophyllin, anthraquinone derivatives, other cinchona alkaloids, and yellow dyes. These absorb light in region of 347.5 mμ.

32.074 Quinine Ethylcarbonate (33)—Official

Weigh quantity of powd. sample equiv. to ca 0.065 g of the alkaloid and transfer to 125 ml erlenmeyer. Add 5 ml alcohol and 15 ml 0.5N NaOH. Place small funnel in neck of flask and heat on steam bath 10 min. Transfer soln to separator. Rinse flask with small portions of H_2O, followed by small portions of HCl (1+3), and add rinsings to soln in separator. Acidify soln with the HCl and then make alk. with NH_4OH. Ext. with four 25 ml portions $CHCl_3$. Wash combined $CHCl_3$ exts with 5 ml H_2O and filter thru cotton pledget into weighed beaker. Evap. to dryness on steam bath, add 5 ml alcohol, and evap. alcohol. Dry 1 hr at 100° and weigh. Det. quinine as in **32.105**(**b**), beginning "dissolve amorphous alkaloid . . ." 1 ml 0.02N H_2SO_4 = 0.00649 g anhyd. quinine, $C_{20}H_{24}O_2N_2$.

Elixirs of Iron, Quinine, and Strychnine (34)—First Action

32.075 APPARATUS

(**a**) *Chromatographic tube.*—Corning No. 38450, or equiv., 20×400 mm, with coarse fritted disk. If unavailable, use plain chromatographic tube of similar dimensions, with retentive cotton pad.

(**b**) *Packing rod.*—Flatten glass rod so that flat end fits smoothly in chromatographic tube.

(**c**) *Test tube.*—25×200 mm, Corning No. 7940 or equiv., marked accurately at 25 ml.

32.076 REAGENTS

(**a**) *Diatomaceous silica.*—Celite 545.

(**b**) *Washed ether.*—Shake equal vols H_2O and ether vigorously 2 min. and discard H_2O. Repeat washing twice and filter ether thru cotton.

(**c**) *Washed chloroform.*—Prep. in same manner as washed ether. Do not use washed $CHCl_3$ after 1 week. Keep in dark, or in amber bottles.

(**d**) *Purified cotton.*—Purify absorbent cotton by washing with several portions each of ether and $CHCl_3$, and drying.

(**e**) *Amalgamated zinc.*—Mix 20 g "30-mesh" Zn with 8 g $HgCl_2$ and then quickly add 20 ml H_2O. Stir mixt. intermittently ca 5 min., pour off aq. phase, and wash amalgam with H_2O until most of soft amalgam is washed off as fine dark suspension. Dry amalgamated Zn in air, break up any clumps, and mix.

(**f**) *Strychine std soln.*—Weigh accurately 50 mg strychnine previously dried 1 hr at 100° and dissolve in 250 ml ca 0.1N HCl. Transfer 10 ml to 200 ml vol. flask, slowly add 10 ml HCl, cool, and dil. to vol. with HCl (1+1). (1 ml = 10 mmg strychnine.) (Strychnine soln in 0.1N HCl will keep at least 1 year; diln in HCl (1+1) should not be used after 3 days.)

(**g**) *Quinine std soln.*—Weigh accurately 100 mg pure quinine previously dried 1 hr at 125° and dissolve in 200 ml 0.1N HCl. To 10 ml of this soln, add 10 ml H_3PO_4, and dil. to 100 ml with 0.1N HCl. (1 ml = 50 mmg quinine.)

32.077 PREPARATION OF CHROMATOGRAPHIC COLUMN

Cover fritted disk with ca $\frac{1}{4}$–$\frac{3}{8}''$ layer (after tamping) of purified cotton (ca 0.4 g). Prep. "wash" layer by mixing 1 g Celite and 1 ml H_2O until all H_2O is absorbed and powder is fluffy. Transfer to chromatographic tube and press down lightly with packing rod. Prep. "trap" layer by mixing 3 g Celite and 3 ml $2N$ HCl until uniform, and transfer in ca 2 equal portions, pressing down each portion lightly with packing rod.

32.078 PREPARATION OF SAMPLE

Evap. 10 ml Elixir of Iron, Quinine, and Strychnine in 250 ml beaker on steam bath. (Evap. elixirs contg non-volatile solvents—glycerine, propylene glycol, etc.—to sirupy residue; evap. elixirs contg volatile solvents to dryness and dissolve residue in 4 ml H_2O instead of 3 ml.) Add 3 ml H_2O, stir, add 2 ml $6N$ HCl, stir, and mix thoroly with 6 g Celite. Transfer to chromatographic tube in 4 or 5 portions, pressing down each portion lightly with packing rod. "Wash" beaker by rubbing with 1 g Celite and add dry "washings" to tube; repeat twice. (It is convenient to use rubber policeman and powder funnel for washing and transferring operation.) Press wad of purified cotton on top of column. Over-all height of column, with ca 20 mm i.d. tube, should be 130–150 mm.

32.079 DETERMINATION OF STRYCHNINE

Add 135 ml washed ether to column and let percolate thru, without pressure. Discard ether soln. Elute strychnine.HCl without pressure, using 150 ml washed $CHCl_3$, collecting eluate in beaker. Reserve column for detn of quinine. Evap. eluate to dryness on steam bath with aid of air current, avoiding prolonged heating. Dissolve residue in 10 ml $0.1N$ HCl with warming. Cool, transfer to 50 ml vol. flask with H_2O, dil. to vol., and mix thoroly.

Transfer aliquot contg 0.1–0.2 mg strychnine (but <12.5 ml) to marked test tube. (Use 5 ml with Elixir of Iron, Quinine, and Strychnine, N.F.) Add equal vol. HCl slowly with mixing, and dil. to 25 ml with HCl (1+1). Add 1.5 g amalgam, place test tube in 25 mm opening on steam bath, and heat 12 min. Remove from steam bath and cool quickly in cold H_2O. Add H_2O to make 25 ml and decant mixed soln as completely as possible into 50 ml erlenmeyer. Add 6 drops (0.3 ml) 0.1% $NaNO_2$, freshly prepd, while gently swirling flask, and mix well. Measure absorbance within 15 min. at 530 mμ, using 1 cm cell and HCl (1+1) as reference soln.

Prep. std curve by adding 5, 10, 15, 20, and 25 ml std strychnine soln in HCl (1+1) to test tubes and dil. to 25 ml with HCl (1+1). Proceed as above, beginning: "Add 1.5 g amalgam . . ." If approx. strychnine content is known, use aliquot contg 0.10–0.20 mg strychnine and prep. std at that concn.

Mg strychnine in aliquot

$$= (A \times \text{mg strychnine in std})/A',$$

where A is absorbance of sample and A' is absorbance of std.

32.080 DETERMINATION OF QUININE

Add 15 ml H_2O to chromatographic tube, apply gentle air pressure, and collect eluate in 250 ml separator. Repeat with five 15 ml portions H_2O. (5 ml of sixth portion should give no test for Cl.) Sep. $CHCl_3$ and wash it in second separator with 20 ml H_2O. Combine aq. layers, filter thru gooch (caution: possible initial foaming) into 200 ml vol. flask, wash, dil. to vol., and det. quinine as in **32.073(b)**. For Elixir of Iron, Quinine, and Strychnine, N.F., use 10 ml aliquot for diln to 100 ml.

Reserpine (*35*)—First Action

32.081 REAGENTS

(a) *Sulfamic acid soln.*—2.5%. Prep. fresh every 2–3 days.

(b) *Alcoholic sodium nitrite soln.*—Dissolve 10 g $NaNO_2$ in 100 ml H_2O. Store in refrigerator. Mix 1 ml of this aq. soln with 50 ml alcohol.

(c) *Reserpine std soln.*—Dissolve 25 mg cryst. reserpine std in ca 40 ml boiling alcohol, cool, and dil. to 100 ml with alcohol. Dil. 10 ml of this stock soln to 50 ml with alcohol. When stored in tightly stoppered brown bottle in dark, solns are stable for weeks.

32.082 DETERMINATION

(a) *Crystalline reserpine.*—Weigh accurately ca 25 mg reserpine, dissolve in ca 40 ml boiling alcohol, cool, and dil. to 100 ml with alcohol. Transfer 10.0 ml to separator contg 50 ml 1% $NaHCO_3$ soln. Ext. with 20, 10, and 10 ml $CHCl_3$, washing each $CHCl_3$ ext. in second separator with 50 ml *2% citric acid soln.* Filter $CHCl_3$ exts thru cotton into 50 ml vol. flask contg 5 ml alcohol, dil. to 50 ml with $CHCl_3$, and mix.

Transfer duplicate 5.0 ml aliquots to 25 ml vol. flasks contg 15 ml alcohol. Transfer duplicate 5.0 ml aliquots of the dil. reserpine std soln to 25 ml vol. flasks contg 10 ml alcohol and 4.5 ml $CHCl_3$. Add 1.0 ml alc. $NaNO_2$ soln to 1 of the stds and to 1 of the sample solns. Add 10 drops HCl to all flasks, swirl, and let stand 30 min. Add 1.0 ml sulfamic acid soln, dil. with alcohol to 25 ml, and mix. Let stand 15 min. and det. absorbances in matched 1 cm cells at 390 mμ against alcohol.

Mg reserpine in sample weighed $= 25 \times (A - A_0)/(S - S_0)$, where A and A_0 are absorbances of nitrite-treated and untreated sample, resp., and S and S_0 are absorbances of corresponding std aliquots.

(b) *Tablets.*—Transfer accurately weighed portion powd. tablets equiv. to ca 5 mg reserpine to 100 ml beaker. Add 20 ml alcohol, cover with watch glass, and heat to simmering. Boil gently 20 min., stirring occasionally, adding small portions of alcohol to maintain vol. Cool to $<50°$, add 10 ml $CHCl_3$, and mix. Filter thru pledget of cotton, and collect filtrate in 50 ml vol. flask. Wash filter and solids with several portions $CHCl_3$. Cool, dil. to 50 ml, and mix. Transfer 25 ml aliquot to separator contg 50 ml 1% $NaHCO_3$. Add 5 ml $CHCl_3$ and shake vigorously. Transfer $CHCl_3$ layer to separator contg 50 ml *2% citric acid soln*, and shake. Repeat extns with two 10 ml portions $CHCl_3$. Filter exts thru cotton and collect in 50 ml vol. flask contg 5 ml alcohol. Proceed as in (a), second par. after dilg to vol. with $CHCl_3$.

Mg reserpine in portion powd. tablets weighed $= 5 \times (A - A_0)/(S - S_0)$.

Reserpine-Rescinnamine Group Alkaloids in *Rauwolfia serpentina* (36)—First Action

32.083 REAGENTS

(a) *1,1,1-Trichloroethane.*—Redistill in all-glass app., collecting fraction boiling at 73–76°.

(b) *Reserpine std soln.*—Dissolve 20.0 mg cryst. reserpine std in 25 ml hot alcohol, cool, and dil. to 50 ml with alcohol. Dil. 5 ml of this soln to 100 ml with alcohol (20 mmg/ml).

(c) *Dilute sulfuric acid.*—0.5*N*. Dissolve ca 30 ml H_2SO_4 in 2 L H_2O.

(d) *Sulfamic acid soln.*—5% aq. soln. Prep. fresh every 2–3 days.

32.084 APPARATUS

Soxhlet extraction apparatus.—Medium size extractor with 250 ml flask and 35×80 mm thimble is most convenient, although smaller app. may be used.

32.085 DETERMINATION

Ext. 2–3 g finely powd. *Rauwolfia serpentina* root or equiv. in powd. tablets in Soxhlet extn app. 4 hr, using ca 100 ml vigorously boiling alcohol. Protect flask and thimble, and all solns of rauwolfia alkaloids, from strong or direct light.

Wash ext. into 100 ml vol. flask with alcohol, cool, dil. to mark, and mix. Transfer 20 ml aliquot to separator contg 200 ml 0.5*N* H_2SO_4, mix, and ext. with three 25 ml portions trichloroethane. Drain lower solvent phase as completely as possible. Wash each trichloroethane ext. in second separator contg 50 ml 0.5*N* H_2SO_4, and discard.

Ext. main aq. soln with 25, 15, 15, 10, 10, and 10 ml $CHCl_3$. Wash each $CHCl_3$ ext. with the acid in second separator, and then with two 10 ml portions 2% $NaHCO_3$ soln in third and fourth separators. Filter $CHCl_3$ exts thru cotton into 100 ml vol. flask contg 10 ml alcohol. Dil. to 100 ml with $CHCl_3$ and mix.

Transfer duplicate 10.0 ml aliquots to 18×150 mm test tubes and mix each with 4 ml alcohol. Add two or three "20-mesh" SiC boiling chips, and heat to boiling in H_2O bath at ca 70°. Raise bath temp. gradually to 100°, or until boiling in tubes *just* stops (avoid prolonged heating in absence of solvent). Wipe outsides of warm tubes, place in vac. desiccator, and evap. to dryness under vac. Dissolve residues by agitating with 5.0 ml alcohol.

Take duplicate 5 ml aliquots of the 20 mmg/ml reserpine std soln, add 2.0 ml 0.5*N* H_2SO_4 to one sample tube and to one std tube (the blanks). To other tubes add 1.0 ml 0.5*N* H_2SO_4 and 1.0 ml 0.3% $NaNO_2$ soln. Mix contents of each tube, and warm in H_2O bath 20 min. at 50–60°. Cool, add 0.5 ml of the sulfamic acid soln to each tube, and mix. Let stand 15 min. and det. absorbances in matched 1 cm cells at 390 mμ against alcohol-H_2O (2+1). Mg reserpine-rescinnamine alkaloids in sample weighed $= 5 \times (A - A_0)/(S - S_0)$, where A and A_0 are the absorbances of nitrite-treated and untreated samples, resp., and S and S_0 are corresponding absorbances for std soln aliquots.

32.086 Strychnine in Liquid Preparations (37)—Official

(Other alkaloids absent. *See* also **32.075–32.079**)

Measure into evapg dish 50 ml sample, or enough to yield at least 0.065 g of the alkaloid, and remove alcohol by evapn. Transfer to separator, add 1 ml NH_4OH, or enough to render soln alk., and proceed as in **32.087**, beginning "Ext. 5 times with $CHCl_3$ . . . "

32.087 Strychnine in Tablets (38)—Official

(Other alkaloids absent)

Count and weigh enough tablets contg ca 0.065 g of the alkaloidal salt and transfer to small beaker. If color on coated tablets interferes with indicator in titrn, wash off without removing strychnine. Add 10 ml HCl (1+7), disintegrate tablets with stirring rod, warm on steam bath ca 10 min., cool, and transfer to separator with not >10 ml H_2O. To remove all strychnine, add to beaker 2 ml NH_4OH (or excess) and 25 ml $CHCl_3$, rinse, and add to separator. Rinse beaker with portions of $CHCl_3$ subsequently to be used for each extn.

Ext. 5 times with $CHCl_3$, using 25, 20, 15, 10, and 5 ml portions, or until alkaloid is completely

extd. Combine first 2 extns in second separator contg absorbent cotton pledget wet with $CHCl_3$ in stem. Wash with 5 ml H_2O contg 1 drop NH_4OH (1+2). When clear, filter $CHCl_3$ into small beaker. Wash each successive $CHCl_3$ ext. with same wash H_2O and filter in similar manner into main portion, finally washing outer surface of separator stem with few ml $CHCl_3$ and adding this also to main portion. Evap. on steam bath, removing dish from bath as last portions evap. to avoid decrepitation.

Add 2–5 ml neutral alcohol, cover beaker, and warm on steam bath to dissolve residue. If necessary, add just enough addnl neutral alcohol to complete soln. Add 2 drops Me red, **32.023(b)**, and titr. with $0.02N$ H_2SO_4 to faint pink. If >2 ml alcohol was used, evap. excess, cool, dil. with 50 ml recently boiled H_2O, and continue titrn with the $0.02N$ H_2SO_4 to faint pink. If preferred, add excess of $0.02N$ H_2SO_4 to alc. soln of the alkaloids, evap. alcohol if necessary as above, and titr. excess acid with $0.02N$ NaOH.

1 ml $0.02N$ $H_2SO_4 = 0.00669$ g $C_{21}H_{22}O_2N_2$, 0.00857 g $(C_{21}H_{22}O_2N_2)_2.H_2SO_4.5H_2O$, or 0.00795 g $C_{21}H_{22}O_2N_2.HNO_3$.

Theobromine and Phenobarbital (*39*)—Official

32.088 REAGENTS

(**a**) *Theobromine std soln.*—1.00 mg/100 ml. Dissolve 100 mg theobromine in H_2SO_4 (1+4), and dil. to 100 ml with this acid. Transfer 5.0 ml aliquot to 500 ml vol. flask, add 200 ml 5% NaOH soln, and cool to room temp. Dil. to mark with H_2O and mix thoroly.

(**b**) *Phenobarbital std soln.*—1.50 mg/100 ml. Dissolve 75.0 mg phenobarbital in $CHCl_3$ and dil. to 100 ml with $CHCl_3$. Dil. 10 ml aliquot to 50 ml with $CHCl_3$. Transfer 10 ml aliquot of latter soln to 100 ml vol. flask, dil. to mark with $CHCl_3$, and mix.

32.089 SEPARATION OF THEOBROMINE AND PHENOBARBITAL

Transfer portion of well-mixed sample contg at least 15 mg phenobarbital to 125 ml separator, add 15 ml 5% NaOH soln, and ext. with three 30 ml portions $CHCl_3$. Wash each $CHCl_3$ ext. with 10 ml 5% NaOH soln in second separator. Discard $CHCl_3$.

Add 30 ml H_2SO_4 (1+4) to alk. mixt. in first separator, cool thoroly, and shake with 50 ml ether. Transfer aq. layer contg dissolved theobromine to second separator, cool, and shake with 40 ml ether. Remove lower phase to third separator and wash with another 40 ml portion ether. Repeat extn thru the 3 separators, using two 40 ml portions H_2SO_4 (1+4) and two 20 ml portions H_2O. Collect aq. exts in 250 ml vol. flask, dil. to mark with H_2O, and mix. Reserve for theobromine detn.

Filter ether solns thru cotton pledget into beaker, washing the 3 separators and filter successively with three 5 ml portions ether. Evap. carefully to dryness, and dissolve residue in $CHCl_3$.

32.090 SPECTROPHOTOMETRIC DETERMINATIONS

(**a**) *Theobromine.*—Pipet aliquot contg 4–8 mg theobromine into 500 ml vol. flask, add 200 ml 5% NaOH soln, and cool to room temp. Dil. to mark with H_2O and mix. Det. absorbance at 274 mμ of this soln, A, and of the std theobromine soln, **32.088(a)**, A', relative to soln prepd by dilg 10 ml of the 5% NaOH soln to 25 ml. Calc. theobromine content of sample.

Mg theobromine in aliquot $= 5.0\ A/A'$.

(**b**) *Phenobarbital.*—Transfer the $CHCl_3$ soln to vol. flask and dil. with $CHCl_3$ to obtain soln contg 20–40 mg phenobarbital/100 ml. Place 5.0 ml in 100 ml vol. flask, dil. to mark with $CHCl_3$, and mix. Transfer 20 ml aliquot of latter soln to separator contg 25 ml NH_4OH (1+24). Treat similarly 20 ml aliquot of the std phenobarbital soln, **32.088(b)**, and 20 ml portion $CHCl_3$ as blank. Shake vigorously at least 1 min., sep., and discard $CHCl_3$. Let aq. ext. stand 30 min. Det. absorbance at 241 mμ of the clear aq. solns of sample, A, and of std, A', relative to blank, using same cell for std and sample. Calc. phenobarbital content of sample.

Mg phenobarbital in final aliquot $= 0.30A/A'$.

In presence of salicylates, proceed as in (**c**).

(**c**) *Phenobarbital in presence of salicylates.*—Prep. chromatographic column as in **32.136**, and adjust flow to 2–4 ml/min.

When $CHCl_3$ just stops flowing from tube, pipet 5 ml of the original $CHCl_3$ soln (**b**), (equiv. to 1–2 mg phenobarbital) into tube, and collect eluate in 100 ml vol. flask. As level of $CHCl_3$ soln reaches top of Celite column, add ca 5 ml $CHCl_3$, and repeat with second $CHCl_3$. wash. Add enough $CHCl_3$ to keep column of solvent 2–5 cm high, and collect ca 95 ml eluate. Wash outside surface of stem with stream of $CHCl_3$ and collect washings in vol. flask. Dil. to 100 ml with $CHCl_3$ and mix thoroly. Det. phenobarbital in eluate as in (**b**), beginning "Transfer 20 ml aliquot of latter soln . . . "

Theobromine in Theobromine-Calcium Salicylate (*40*)—Official

32.091 *Method I.*

Dry ca 0.5 g sample at 110° to constant wt. Weigh 0.2 g dried substance into g-s. 100 ml vol. flask, add 2 ml HOAc, and warm on steam bath. Add 10 ml boiling H_2O and shake until dissolved,

adding more boiling H_2O if necessary. Cool soln to room temp. (Soln should be clear or nearly so.) Add 50 ml 0.1*N* I, 20 ml satd NaCl soln, and 2 ml HCl. Shake well and dil. to vol. with H_2O. Shake again and let stand overnight. Filter, discarding first 10 ml filtrate. Titr. 50 ml filtrate with 0.1*N* $Na_2S_2O_3$, using starch soln, **4.004(f)**, as indicator. 1 ml 0.1*N* I = 0.00451 g theobromine, $C_7H_8O_2N_4$.

Method II.—In Tablets (41)

32.092 INDICATOR

Phenol red indicator.—Triturate 0.1 g phenol red in agate mortar with 15 ml 0.02*N* NaOH until dissolved and dil. soln with recently boiled H_2O to 200 ml.

32.093 DETERMINATION

Place 0.5 g powd. tablets, or 0.4 g powder, or 0.2 g theobromine alkaloid, in 300 ml beaker and add 100 ml H_2O. Warm moderately over flame and add 15 ml ca 0.1*N* H_2SO_4. Heat to boiling to insure complete soln and to remove CO_2. Cool to room temp. Add 1.5 ml of the phenol red indicator and make slightly alk. with ca 0.1*N* NaOH (violet-red); then titr. carefully to acid reaction with 0.1*N* H_2SO_4 (yellow). To this soln add 25 ml (an excess) neutral 0.1*N* $AgNO_3$, **42.025**, and titr. liberated HNO_3 immediately with 0.1*N* NaOH to distinct violet-red. Titr. cautiously dropwise with constant stirring near end point. 1 ml 0.1*N* NaOH = 0.01802 g $C_7H_8O_2N_4$.

32.094 Theophylline *(42)*—**Official**

(Applicable to solns and tablets)

Weigh 0.2–0.3 g theophylline (or equiv. of powd. tablets), or measure equiv. quantity of soln, into separator. Add 5 ml 0.5*N* NaOH and shake gently until alkaloid dissolves. Add strip of litmus paper and enough 0.5*N* HCl from buret to produce distinct acid reaction; then add 0.5 ml excess. Add 30 ml $CHCl_3$-isopropyl alcohol (3+1) and shake 1 min. Let settle and drain lower layer into second separator contg 10 ml H_2O acidified with HCl. Shake well, let settle, and filter solvent into weighed flask thru cotton pledget placed in stem of funnel.

Repeat extn with 6 more 20 ml portions of the $CHCl_3$-isopropyl alcohol mixt., wash each portion thru second separator, and pass solvent thru filter into weighed flask. Insure complete extn by seventh shaking with 10 ml of the solvent and evapn of washed solvent in sep. container. Recover most of solvent and evap. remainder on steam bath while rotating container in inclined position. Add 2 ml absolute ether to residue and evap. (cautiously to avoid spattering). Dry residue at 80° to constant wt and weigh as anhyd. theophylline. $C_7H_8O_2N_4 \times 1.10 = C_7H_8O_2N_4.H_2O$.

ANTIPYRETIC DRUGS

Acetanilid and Acetophenetidin (Phenacetin) *(43)*

32.095 *Qualitative Test for Acetophenetidin—Procedure*

To 1–2 mg sample in test tube add drop of HOAc, 0.5 ml H_2O, and 1 ml 0.1*N* I; warm mixt. to ca 40° and add drop of HCl. If acetophenetidin alone is present, its periodide seps almost immediately in form of reddish brown leaflets or needle-like crystals. If sample consists largely of acetanilid, sepn takes place on cooling and shaking the liquid. In presence of considerable acetanilid, periodide first seps as minute, oily globules, which on vigorous shaking gradually become cryst. By this test as little as 0.5 mg acetophenetidin, if alone, may be detected in form of its characteristic periodide.

Quantitative Methods—Official

32.096 REAGENTS

(**a**) *Sodium thiosulfate std soln.*—Dissolve 30 g $Na_2S_2O_3.5H_2O$ in recently boiled, cooled H_2O and dil. to ca 1 L. Stdze this soln against reagent I as follows: Weigh accurately ca 0.3 g of I in small glass capsule provided with tight-fit glass cap or stopper. Place capsule in 200 ml erlenmeyer contg 0.5 g KI dissolved in 1–2 ml H_2O. After complete soln, dil. with 10 ml H_2O and titr. with the $Na_2S_2O_3$ soln, using 1 or 2 drops starch indicator, **4.004(f)**.

(**b**) *Iodine std soln.*—Dissolve 40 g KI in min. quantity of H_2O, add 30 g I, and after soln dil. to ca 1 L. Stdze against the std $Na_2S_2O_3$ soln.

32.097 DETERMINATION

(**a**) *Acetophenetidin.*—(1) Volumetric.—Place 0.2 g of the acetanilid-acetophenetidin mixt. in 50 ml lipped erlenmeyer, add 2 ml HOAc, heat gently over wire gauze to complete soln, and dil. with 40 ml H_2O previously warmed to 70°. Transfer clear liquid with two 10 ml portions H_2O at 40° to g-s. 100 ml vol. flask contg 25 ml of the std I soln warmed to 40°. Stopper, mix thoroly by rotating liquid, add 3 ml HCl, continue rotating liquid until crystn begins, and then set aside to cool. (If ratio of acetophenetidin to acetanilid is equal to or >1, cryst. scales form almost immediately on addn of acid. As proportion of acetanilid increases, however, periodide tends to remain in liquid state. Gentle agitation or rotation of flask in warm H_2O at not >40° hastens formation of crystals.) When contents are at room temp., fill flask with H_2O to within 2 or 3 ml of mark, mix thoroly by rotation, and let stand overnight. Dil. to mark with H_2O, mix thoroly, let stand 30 min., and filter thru 5.5 cm dry, closely fitted filter into 50 ml vol. flask, rejecting ca 15 ml first filtrate

but reserving it for recovery of acetanilid. Transfer 50 ml aliquot to 200 ml erlenmeyer and titr. excess I with the std $Na_2S_2O_3$ soln. Formula of the pptd periodide is $(C_2H_5O.C_6H_4NH.COCH_3)_2HI.I_4$. 1 ml 0.25*N* I = 0.0224 g $C_{10}H_{13}O_2N$.

(2) Gravimetric.—Filter off periodide, preferably by suction; wash with 10–15 ml of the std I soln; and transfer ppt, together with filter and any particles of ppt remaining in vol. flask, to separator, using not >50 ml H_2O. Remove both free and added I with few small crystals of Na_2SO_3 and ext. liquid with three 50 ml portions $CHCl_3$, washing each portion subsequently in second separator with 5 ml H_2O. After washing and clearing, filter $CHCl_3$ soln thru small dry filter into 200 ml erlenmeyer, evap. most of $CHCl_3$, transfer residual soln (5–10 ml) with little $CHCl_3$ to small weighed beaker, evap. to dryness on steam bath, cool, and weigh.

(**b**) *Acetanilid.*—If combined wt of the acetanilid-acetophenetidin mixt. is known, det. wt acetanilid by difference; or det. it directly from second aliquot of filtrate from the acetophenetidin periodide (**a**) as follows:

Pipet 25–30 ml of the clear liquid into separator and decolorize with solid Na_2SO_3; add solid $NaHCO_3$ in slight excess and then 1 or 2 drops Ac_2O. Ext. with three 60 ml portions $CHCl_3$, passing $CHCl_3$ soln thru small dry filter into 200 ml erlenmeyer, and distill $CHCl_3$ with gentle heating to ca 20 ml. Add 10 ml H_2SO_4 (1+9) and digest on steam bath until vol. is reduced one-half. Add 20 ml H_2O and continue digestion 1 hr. Add second 20 ml portion H_2O and 10 ml HCl, and titr. very slowly, dropwise, with 0.1*N* KBr-$KBrO_3$ soln, **32.128**, to faint yellow. While adding this reagent, rotate flask enough to agglomerate pptd tribromoaniline. 1 ml 0.1*N* KBr-$KBrO_3$ = 0.00225 g C_8H_9ON.

If prepn contains antipyrine or caffeine, or both, in addn to acetanilid and acetophenetidin, proceed as follows: (1) Digest mixt. by heating with H_2SO_4 (1+9) to convert acetophenetidin and acetanilid to phenetidin and aniline sulfates, resp., **32.100**(**a**); (2) remove caffeine and antipyrine by extn with $CHCl_3$ and sep. them as in **32.145**; (3) regenerate acetophenetidin and acetanilid by treating soln of corresponding sulfates with solid $NaHCO_3$ in slight excess and few drops of Ac_2O, and ext. with $CHCl_3$. Evap. $CHCl_3$, dry, and weigh residue. Sep. constituents as in simple mixts.

Acetanilid and Caffeine (*44*)—Official

32.098 REAGENTS

(**a**) *Bromide-bromate std soln.*—Dissolve 14 g $KBrO_3$ and 55 g KBr in H_2O. Dil. to 1 L and stdze by one of following methods: (1) Weigh accurately ca 0.4 g recrystd and dried acetanilid, and proceed as in **32.097**(**b**), beginning "Add 10 ml H_2SO_4 (1+9) . . . " (2) Transfer 10 ml soln to g-s. flask and add 25 ml H_2O, 5 ml 16.5% KI soln, and 5 ml HCl. Shake thoroly and titr. liberated I with 0.1*N* $Na_2S_2O_3$, using starch soln, **4.004**(**f**) as indicator.

(**b**) *Iodine soln.*—Dissolve 2 g I and 6 g KI in H_2O, and dil. to 100 ml.

Treat all corks used in distn with $CHCl_3$.

32.099 PREPARATION OF SOLUTION

(**a**) *Solid samples.*—Weigh 0.3–0.5 g powd. sample or, if preferred, quantity equal to, or multiple of, av. unit dose (previously detd by weighing collectively 20 or more such doses). Transfer to separator, add 50 ml $CHCl_3$ and 20 ml H_2O, shake vigorously, and after clearing, drain lower layer thru small dry filter into 250 ml beaker. Repeat extn with two addnl 50 ml portions $CHCl_3$. Recover any caffeine-acetanilid mixt. seen around apex of delivery tube of separator, edge of filter, and tip of separator by careful washing with $CHCl_3$, and add these washings to main portion. Evap. combined $CHCl_3$ exts to ca 10 ml.

If caffeine is present, as free alkaloid or in other readily extractable form, extn may, if preferred, be made on filter paper by washing with successive 5–10 ml portions $CHCl_3$ (30–50 ml is usually enough) until extn is complete, as indicated by absence of any residue after evapn of small portion of last washing.

(**b**) *Liquid samples.*—With dil. alc. solns, evap. measured quantity on steam bath until most of alcohol is expelled, or take aliquot of residue from an alcohol detn and transfer to separator by pouring and rinsing with min. quantity of H_2O so that final vol. does not greatly exceed 20 ml. To avoid loss of acetanilid by hydrolysis during evapn, add little solid $NaHCO_3$ and drop of Ac_2O. If prepn contains other alkaloids, acidify with few drops of H_2SO_4 (1+9) immediately after acetylation to retain such basic material in aq. soln. Add 50 ml $CHCl_3$, shake vigorously, and after clearing, drain the $CHCl_3$ layer thru filter into 250 ml beaker. Repeat extn with two addnl 50 ml portions $CHCl_3$ and distill combined $CHCl_3$ washings to ca 10 ml.

32.100 DETERMINATION

(**a**) *Caffeine.*—Treat $CHCl_3$ soln, **32.099**, with 10 ml H_2SO_4 (1+9) and digest on steam bath until vol. is reduced to 5 ml. Add 10 ml H_2O and continue digestion until vol. is again reduced to 5 ml. (Dilg and evapg must be repeated until odor of HOAc can no longer be detected in vapors.)

Cool and transfer to separator with min. of H_2O. (Final vol. should not greatly exceed 20 ml.) Add 50 ml $CHCl_3$, ext. in usual way, and after clearing, drain lower layer thru small dry filter into 200 ml erlenmeyer. Repeat extn with two 50 ml portions $CHCl_3$. Evap. combined exts to ca 10 ml, finally transferring residual liquid, by washing with $CHCl_3$, to weighed beaker or crystg dish. Let soln evap. spontaneously, or by gentle heat and air blast, to apparent dryness. Cool, and let stand in open until wt becomes constant.

From prepns contg powd. cinnamon, celery seed, ginger, or other vegetable products, $CHCl_3$ exts, in addn to caffeine and acetanilid, certain oils, fats, waxes, resins, pigments, and other substances. After caffeine-acetanilid mixt. has been digested, these oils, etc., appear either in suspension or soln and contaminate the caffeine. Remove any suspended impurities by filtering thru small moistened filter immediately after hydrolysis and prior to extn with $CHCl_3$.

If recovered caffeine is deeply colored or contaminated with foreign matter, purify as follows: Dissolve in H_2SO_4 (ca 5 ml 0.2*N* acid/100 mg caffeine); filter, if necessary, thru moistened filter; add 1 ml 9*N* H_2SO_4 and enough I reagent, **32.098(b)**, to color supernatant deep claret; stir, and let stand 1 hr, preferably in refrigerator. Filter and wash periodide with few ml I soln; transfer both filter and ppt to separator, using not >20 ml H_2O; and decolorize with crystal of Na_2SO_3. Ext. with three 50 ml portions $CHCl_3$ and proceed as above.

(**b**) *Acetanilid*.—(1) Transfer soln of aniline sulfate remaining in separator to erlenmeyer used for hydrolysis and heat 10 min. on steam bath to expel all traces of $CHCl_3$. Wash filter that was used in drying the $CHCl_3$ soln of caffeine with 5 ml H_2O, adding washings to main soln of aniline sulfate. Add 10 ml HCl and titr. with std KBr-$KBrO_3$ soln, **32.098(a)**, to faint yellow, rotating flask enough to agglomerate pptd tribromoaniline. 1 ml 0.5*N* KBr-$KBrO_3$ = 0.0113 g C_8H_9ON.

(2) Add excess of the std KBr-$KBrO_3$ soln to soln of aniline sulfate obtained under (**b**)(1) and titr. the excess with 0.1*N* $Na_2S_2O_3$ after addn of 5 ml KI soln and starch soln, **4.004(f)**, as indicator. 1 ml 0.1*N* KBr-$KBrO_3$ = 0.00225 g acetanilid.

(**c**) *Other ingredients*.—To det. $NaHCO_3$ also, which often appears as the $CHCl_3$-insol. residue, titr. such residue with std acid, using Me orange. The bicarbonate may also be detd by igniting original sample (if talc is absent) or $CHCl_3$-insol. residue with H_2SO_4 and weighing resulting Na_2SO_4. $Na_2SO_4 \times 1.183 = NaHCO_3$.

If "acetanilid compound" is combined with NaBr, the Br in absence of other halides may be detd volumetrically as in 6.068. 1 ml 0.1*N* $AgNO_3$ = 0.01029 g NaBr.

Acetanilid, Caffeine, and Codeine (*45*)—Official

32.101 PREPARATION OF SOLUTION

Transfer to separator one or more average unit doses (ca 0.2 g acetanilid) of powd. sample; add 20 ml H_2O, 50 ml $CHCl_3$, and 10 drops H_2SO_4 (1+9); and ext. in usual way. After clearing, wash solvent in second separator with 5 ml H_2O and transfer to 250 ml beaker. Repeat extn with two 50 ml portions $CHCl_3$, finally evapg combined $CHCl_3$ solns by gentle heat to ca 10 ml. Test for complete extn.

32.102 DETERMINATION

(**a**) *Acetanilid and caffeine*.—Treat $CHCl_3$ residue, **32.101**, as in **32.100**.

(**b**) *Codeine*.—Combine wash H_2O used in second separator in **32.101** with the soln of codeine sulfate. Add excess of solid $NaHCO_3$, ext. with successive portions of 30, 25, 20, 15, and 10 ml $CHCl_3$, wash combined $CHCl_3$ exts with 5 ml H_2O in second separator, and pass thru dry filter into 200 ml erlenmeyer. Test for complete extn, evap. to apparent dryness in small weighed beaker on steam bath, add few drops of alcohol and equal quantity of H_2O to amorphous residue, and evap. again. Finally cool and let usually cryst. product stand until wt becomes constant. Check this result volumetrically by dissolving residue in 3–5 ml neutral alcohol and titrg with 0.02*N* H_2SO_4 to faint red, using Me red, **32.023(b)**. 1 ml 0.02*N* H_2SO_4 = 0.00599 g $C_{18}H_{21}O_3N$, 0.00635 g $C_{18}H_{21}O_3N.H_2O$, or 0.00849 g $C_{18}H_{21}O_3N.H_3PO_4.1.5\ H_2O$.

Quantity of codeine found by wt is usually slightly greater than that detd by titrn. To insure max. accuracy in volumetric operations, check strength of std acid used by titrn against pure codeine.

Acetanilid, Caffeine, and Quinine (*45*)—Official

32.103 REAGENT

Bromocresol purple soln.—Triturate 0.1 g bromocresol purple in agate mortar with 9 ml 0.02*N* NaOH. When dissolved, dil. with H_2O to 200 ml, and filter if necessary. Soln should be deep orange to red. If it is purple, addn of not >0.5 ml 0.02*N* acid should make it red. If it is yellow, addn of not >0.5 ml 0.02*N* alkali should make it red.

32.104 PREPARATION OF SOLUTION

See 32.101.

32.105 DETERMINATION

(**a**) *Acetanilid and caffeine*.—*See* **32.100**.

(**b**) *Quinine*.—Combine wash H_2O used in second separator in **32.101** with the soln of quinine bisulfate, add slight excess of NH_4OH, and ext. with three 50 ml portions $CHCl_3$. Wash each

acid may be checked by Br method, **32.129(b)**, or by double titrn method, **32.132**.

(b) *Acetophenetidin and caffeine.*—Evap. $CHCl_3$ soln contg the acetophenetidin and caffeine on steam bath and transfer, when vol. reaches 5–10 ml, to 100 ml beaker, using small portions of $CHCl_3$. Evap. again to ca 5 ml and add 10 ml 2% H_2SO_4. Insert stirring rod and heat mixt. on bath until all $CHCl_3$ evaps, stirring occasionally. Cool to room temp. and decant without suction thru tared gooch previously dried to constant wt at 100°. Collect filtrate in 150 ml beaker, retaining as much acetophenetidin as possible in beaker. Rinse sides of beaker contg acetophenetidin with 5–10 ml $CHCl_3$, add 10 ml 2% H_2SO_4, and heat on bath as before until all $CHCl_3$ evaps. Cool, and decant thru same crucible as before. Repeat process with another 10 ml portion of the H_2SO_4, and finally wash acetophenetidin quantitatively into crucible with H_2O. Wash beaker and crucible with H_2O until filtrate measures ca 75 ml. Dry crucible at 100° and weigh acetophenetidin.

To filtrate contg the caffeine and small quantity of acetophenetidin still in soln (ca 0.075 g), add 5 ml H_2SO_4 (1+9) and evap. on steam bath to ca 10 ml. Transfer with small portions of H_2O to 50 ml erlenmeyer previously marked for vols of 5 and 10 ml. Proceed as in **32.123(a)**, bearing in mind that hydrolysis must be continued until no odor of HOAc is present. Hydrolysis is hastened somewhat if flask is allowed to hang in the steam by wire wrapped around its neck so that mouth of flask is ca level with surface of bath (ca 3 evaps are usually enough). Wt acetophenetidin obtained + wt acetophenetidin collected in gooch = total acetophenetidin.

Chromatographic Method (50)—First Action

32.113 REAGENTS

(a) *Sodium bicarbonate soln.*—1*M*. Dissolve 4.2 g $NaHCO_3$ in 48 ml H_2O.

(b) *Washed ether.*—Wash USP grade ether with equal vol. H_2O in separator. Filter thru paper, rejecting first 15 ml. Use within 3 days. Approx. 70 ml required for each sample.

(c) *Chloroform.*—USP. Absorbance against H_2O at 276 mμ not >0.050. Use same lot thruout.

(d) *Washed chloroform.*—Wash $CHCl_3$ with equal volume H_2O in separator. Filter thru paper, rejecting first 15 ml. Use within 3 days. Use same lot thruout. Approx. 700 ml required for stds, and 170 ml for each sample.

(e) *Isooctane.*—Absorbance against H_2O at 286 mμ not >0.050. Use same lot thruout.

(f) *Acetophenetidin std soln.*—7 mg/100 ml. Dissolve 70.0 mg pure acetophenetidin in $CHCl_3$ and dil. to 100 ml with isooctane. Dil. 10 ml aliquot to 100 ml with isooctane.

(g) *Caffeine std soln.*—1.4 mg/100 ml. Dissolve 140.0 mg caffeine in washed $CHCl_3$ and dil. to 100 ml. Dil. 10 ml aliquot to 100 ml; dil. 10 ml aliquot of this soln to 100 ml with washed $CHCl_3$.

(h) *Acetylsalicylic acid std soln.*—5 mg/100 ml. Dissolve 100.0 mg acetylsalicylic acid in washed $CHCl_3$ and dil. to 100 ml. To 5 ml aliquot add 1.0 ml HOAc and dil. to 100 ml with washed $CHCl_3$. Prep. fresh daily.

(i) *Salicylic acid std soln.*—2.5 mg/100 ml. Dissolve 100.0 mg salicylic acid in washed $CHCl_3$ and dil. to 100 ml. Dil. 25 ml aliquot to 100 ml; to 10 ml aliquot of this soln, add 1.0 ml HOAc and dil. to 100 ml with washed $CHCl_3$.

32.114 APPARATUS

Use app. of **32.133**. Packing rod may be of stainless steel, Al, or glass.

32.115 PREPARATION OF SAMPLE

Weigh powd. sample contg ca 100 mg acetylsalicylic acid and transfer to 100 ml vol. flask. Add 60 ml $CHCl_3$ and shake well. Add 0.2 ml HOAc and dil. to vol. with $CHCl_3$.

32.116 PREPARATION OF CHROMATOGRAPHIC COLUMN

Pack loosely small amount of fine glass wool in base of chromatographic tube so as to support Celite above, but not cause irregularity in thickness of Celite layer.

To 2.0 g Celite 545 in 100 ml beaker, or glass mortar, add 2.0 ml H_2SO_4 (1+9). Mix well with metal spatula. Transfer to chromatographic tube, and with packing rod compress lightly to uniform mass. Mix 2.0 g Celite with 2.0 ml 1*M* $NaHCO_3$ and place in column above acid layer. Wash column with 15–20 ml washed ether and discard washings.

32.117 SEPARATION

(Use washed ether and washed $CHCl_3$ thruout, except for dissolving acetophenetidin residue.)

(a) *Acetophenetidin.*—Dil. 5 ml aliquot prepd sample soln with 20 ml ether and pass thru column, receiving eluate in 100 or 150 ml beaker. After soln has passed into absorbent, wash with five 5 ml portions ether, letting each portion pass into absorbent before adding next. Wash tip of outlet with $CHCl_3$ and evap. total eluate to dryness by gentle heating on steam bath with air current. Dissolve acetophenetidin residue in 5 ml USP $CHCl_3$ and dil. with isooctane to 50 ml.

(b) *Caffeine.*—Immediately after passage of last portion of ether thru column, replace beaker with 50 ml vol. flask. Pass 48 ml $CHCl_3$ thru column, wash tip with $CHCl_3$, and dil. eluate to vol. This fraction contains caffeine.

(c) *Acetylsalicylic acid and salicylic acid.*—

and decant clear liquid thru weighed gooch, retaining most of ppt, tetraiodophenylenequinone $(C_6H_2I_2O)_2$, in flask. To latter add 50 ml boiling H_2O, digest 10 min. on steam bath, filter, and gradually wash all ppt into gooch, using for this purpose and final washings ca 200 ml hot H_2O. Dry ppt to constant wt at 100°. $(C_6H_2I_2O)_2 \times 0.4656 = NaC_7H_5O_3$.

Note: If mixt. contains antipyrine or caffeine or both, these substances will appear with acetanilid in first $CHCl_3$ ext., and may be detd as in remarks following **32.145(b)**. If acetanilid is replaced by acetophenetidin in mixt., general procedure would not be materially altered, acetophenetidin being weighed directly after recovery from its washed $CHCl_3$ soln sepd from the Na salicylate. If, instead of Na salicylate, mixt. contains the free acid or its NH_4 salt, add larger quantity of $NaHCO_3$ prior to extn with $CHCl_3$ to insure fixation of salicylic acid.

In analysis of mixt. of caffeine, acetanilid, Na salicylate, and codeine, following procedure is recommended: (1) Extn of caffeine, acetanilid, and salicylic acid from acidified soln, (2) washing $CHCl_3$ soln with aq. Na_2CO_3 for recovery of salicylic acid, preliminary to its treatment with I soln; (3) sepn of caffeine and acetanilid as in **32.100(a)**; and (4) recovery of codeine from soln of its sulfate after treatment with $NaHCO_3$ and $CHCl_3$.

Acetophenetidin, Acetylsalicylic Acid, and Caffeine (APC)

(For antihistamines in combination with APC—see **32.143**)

32.110 *Method I. (For Acetylsalicylic Acid Only) (48)—Official*

Det. av. wt of number of tablets and reduce to fine powder. Weigh ca 0.2 g powder, transfer to separator with ca 25 ml H_2O, and ext. with repeated portions of $CHCl_3$; ca 6 extns with 30, 25, 20, 10, 10, and 5 ml are generally required. Test final extn by evapg small portion on steam bath to dryness.

Collect $CHCl_3$ fractions in separator and drain thru cotton pledget in stem into 200 ml erlenmeyer. Wash separator twice with 5 ml portions $CHCl_3$, passing this thru the cotton and leaving any H_2O that may have sepd in separator. Add $CHCl_3$ washings to flask and evap. $CHCl_3$ on steam bath to ca 2 ml. Add 10 ml H_2SO_4 (1+9), connect with reflux condenser, and digest 30 min., partially immersing flask in boiling H_2O bath.

Cool, and transfer to separator, rinsing condenser with $CHCl_3$ and using min. quantity of H_2O for transfer, so that final vol. does not greatly exceed 20 ml. Ext. caffeine and salicylic acid with 6 portions of $CHCl_3$, using 30, 25, 20, 15, 10, and 10 ml. Collect these fractions in separator, add 20 ml H_2O and 1 g Na_2CO_3, and shake thoroly. Drain $CHCl_3$ into another separator and wash twice more with 15 and 10 ml H_2O. Reject $CHCl_3$, and combine Na_2CO_3 soln and wash H_2O in 200 ml erlenmeyer. Heat on steam bath to expel traces of $CHCl_3$ and dil. to 100 ml with H_2O; then add slowly 25–40 ml strong I soln (ca 0.2*N*), enough to insure excess during digestion, and digest 1 hr on steam bath.

Remove free I with few drops of $Na_2S_2O_3$ soln. Decant clear soln thru weighed gooch, retaining most of ppt in flask. To latter add 50 ml boiling H_2O, digest 10 min. on steam bath, filter, and gradually wash all ppt into the gooch, using altogether ca 200 ml hot H_2O. Dry to constant wt at 100° and weigh ppt of tetraiodophenylenequinone $(C_6H_2I_2O)_2$. Wt ppt $\times 0.4016$ = total salicylic acid. If free salicylic acid is present, **32.127**, deduct from total; difference $\times 1.304$ = wt acetylsalicylic acid.

Method II. (49)—Official

32.111 REAGENTS

(**a**) *Dilute sulfuric acid soln.*—2%. Pour 6.0 ml H_2SO_4 into 500 ml H_2O.

(**b**) *Sodium bicarbonate soln.*—Freshly prepd. Add 3 g $NaHCO_3$ to 45 ml H_2O previously cooled to 15° or lower. Stir until dissolved and add 2–3 drops HCl (1+3).

32.112 DETERMINATION

(**a**) *Acetylsalicylic acid.*—Make detn as soon as possible to prevent any hydrolysis in $NaHCO_3$ soln.

Weigh enough powd. sample to contain at least 0.04 g caffeine, transfer to separator contg ca 10 ml H_2O cooled to 15° or lower, and shake thoroly. Add 15 ml of the cooled $NaHCO_3$ soln slowly to prevent mechanical loss due to effervescence and immediately ext. with successive portions of $CHCl_3$. Usually 5 extns with ca 30 ml portions $CHCl_3$ are enough. Extn is complete when final ext. evapd to dryness leaves negligible residue.

Wash each portion of $CHCl_3$ thru second separator contg 2 ml of the cold $NaHCO_3$ soln and filter thru cotton moistened with $CHCl_3$. Set aside combined $CHCl_3$ exts contg caffeine and acetophenetidin for later treatment. Transfer wash H_2O in second separator to soln in first separator, rinsing several times with small portions of H_2O. Acidify combined $NaHCO_3$ solns with HCl (1+1) and ext. acetylsalicylic acid by shaking with successive portions of $CHCl_3$, filtering each portion thru funnel contg cotton pledget moistened with $CHCl_3$ (usually 5 extns are enough).

Evap. combined $CHCl_3$ exts on steam bath with aid of fan or gentle air blast to ca 10 ml. Transfer to suitable small tared container with $CHCl_3$ and evap. to dryness with aid of fan or gentle air blast without heat. Dry in desiccator overnight and weigh as acetylsalicylic acid. Extd acetylsalicylic

acid may be checked by Br method, **32.129(b)**, or by double titrn method, **32.132**.

(b) *Acetophenetidin and caffeine.*—Evap. $CHCl_3$ soln contg the acetophenetidin and caffeine on steam bath and transfer, when vol. reaches 5–10 ml, to 100 ml beaker, using small portions of $CHCl_3$. Evap. again to ca 5 ml and add 10 ml 2% H_2SO_4. Insert stirring rod and heat mixt. on bath until all $CHCl_3$ evaps, stirring occasionally. Cool to room temp. and decant without suction thru tared gooch previously dried to constant wt at 100°. Collect filtrate in 150 ml beaker, retaining as much acetophenetidin as possible in beaker. Rinse sides of beaker contg acetophenetidin with 5–10 ml $CHCl_3$, add 10 ml 2% H_2SO_4, and heat on bath as before until all $CHCl_3$ evaps. Cool, and decant thru same crucible as before. Repeat process with another 10 ml portion of the H_2SO_4, and finally wash acetophenetidin quantitatively into crucible with H_2O. Wash beaker and crucible with H_2O until filtrate measures ca 75 ml. Dry crucible at 100° and weigh acetophenetidin.

To filtrate contg the caffeine and small quantity of acetophenetidin still in soln (ca 0.075 g), add 5 ml H_2SO_4 (1+9) and evap. on steam bath to ca 10 ml. Transfer with small portions of H_2O to 50 ml erlenmeyer previously marked for vols of 5 and 10 ml. Proceed as in **32.123(a)**, bearing in mind that hydrolysis must be continued until no odor of HOAc is present. Hydrolysis is hastened somewhat if flask is allowed to hang in the steam by wire wrapped around its neck so that mouth of flask is ca level with surface of bath (ca 3 evaps are usually enough). Wt acetophenetidin obtained + wt acetophenetidin collected in gooch = total acetophenetidin.

Chromatographic Method (50)—First Action

32.113 REAGENTS

(a) *Sodium bicarbonate soln.*—1*M*. Dissolve 4.2 g $NaHCO_3$ in 48 ml H_2O.

(b) *Washed ether.*—Wash USP grade ether with equal vol. H_2O in separator. Filter thru paper, rejecting first 15 ml. Use within 3 days. Approx. 70 ml required for each sample.

(c) *Chloroform.*—USP. Absorbance against H_2O at 276 mμ not >0.050. Use same lot thruout.

(d) *Washed chloroform.*—Wash $CHCl_3$ with equal volume H_2O in separator. Filter thru paper, rejecting first 15 ml. Use within 3 days. Use same lot thruout. Approx. 700 ml required for stds, and 170 ml for each sample.

(e) *Isooctane.*—Absorbance against H_2O at 286 mμ not >0.050. Use same lot thruout.

(f) *Acetophenetidin std soln.*—7 mg/100 ml. Dissolve 70.0 mg pure acetophenetidin in $CHCl_3$ and dil. to 100 ml with isooctane. Dil. 10 ml aliquot to 100 ml with isooctane.

(g) *Caffeine std soln.*—1.4 mg/100 ml. Dissolve 140.0 mg caffeine in washed $CHCl_3$ and dil. to 100 ml. Dil. 10 ml aliquot to 100 ml; dil. 10 ml aliquot of this soln to 100 ml with washed $CHCl_3$.

(h) *Acetylsalicylic acid std soln.*—5 mg/100 ml. Dissolve 100.0 mg acetylsalicylic acid in washed $CHCl_3$ and dil. to 100 ml. To 5 ml aliquot add 1.0 ml HOAc and dil. to 100 ml with washed $CHCl_3$. Prep. fresh daily.

(i) *Salicylic acid std soln.*—2.5 mg/100 ml. Dissolve 100.0 mg salicylic acid in washed $CHCl_3$ and dil. to 100 ml. Dil. 25 ml aliquot to 100 ml; to 10 ml aliquot of this soln, add 1.0 ml HOAc and dil. to 100 ml with washed $CHCl_3$.

32.114 APPARATUS

Use app. of **32.133**. Packing rod may be of stainless steel, Al, or glass.

32.115 PREPARATION OF SAMPLE

Weigh powd. sample contg ca 100 mg acetylsalicylic acid and transfer to 100 ml vol. flask. Add 60 ml $CHCl_3$ and shake well. Add 0.2 ml HOAc and dil. to vol. with $CHCl_3$.

32.116 PREPARATION OF CHROMATOGRAPHIC COLUMN

Pack loosely small amount of fine glass wool in base of chromatographic tube so as to support Celite above, but not cause irregularity in thickness of Celite layer.

To 2.0 g Celite 545 in 100 ml beaker, or glass mortar, add 2.0 ml H_2SO_4 (1+9). Mix well with metal spatula. Transfer to chromatographic tube, and with packing rod compress lightly to uniform mass. Mix 2.0 g Celite with 2.0 ml 1*M* $NaHCO_3$ and place in column above acid layer. Wash column with 15–20 ml washed ether and discard washings.

32.117 SEPARATION

(Use washed ether and washed $CHCl_3$ thruout, except for dissolving acetophenetidin residue.)

(a) *Acetophenetidin.*—Dil. 5 ml aliquot prepd sample soln with 20 ml ether and pass thru column, receiving eluate in 100 or 150 ml beaker. After soln has passed into absorbent, wash with five 5 ml portions ether, letting each portion pass into absorbent before adding next. Wash tip of outlet with $CHCl_3$ and evap. total eluate to dryness by gentle heating on steam bath with air current. Dissolve acetophenetidin residue in 5 ml USP $CHCl_3$ and dil. with isooctane to 50 ml.

(b) *Caffeine.*—Immediately after passage of last portion of ether thru column, replace beaker with 50 ml vol. flask. Pass 48 ml $CHCl_3$ thru column, wash tip with $CHCl_3$, and dil. eluate to vol. This fraction contains caffeine.

(c) *Acetylsalicylic acid and salicylic acid.*—

Immediately replace receiver with 100 ml vol. flask. Pass soln of 0.5 ml HOAc in 5 ml $CHCl_3$ thru column, followed by 90–92 ml 1% soln of HOAc in $CHCl_3$. Wash tip with $CHCl_3$ and dil. eluate to vol.

32.118 DETERMINATION

Immediately det. absorbance of the acid fraction and of acetylsalicylic and salicylic acid std solns at 280 mμ and 310 mμ against 1% HOAc in $CHCl_3$. Det. absorbance of acetophenetidin fraction and std at 286 mμ against isooctane-USP $CHCl_3$ (9+1) and that of caffeine fraction and std at 276 mμ against washed $CHCl_3$ blank.

Calc. quantity of each ingredient in sample. Acetylsalicylic and salicylic acids may be calcd by successive approximations as follows: Attributing entire absorbance at 310 mμ to salicylic acid, use ratio of salicylic acid std readings at the 2 wavelengths to calc. absorbance due to salicylic acid at 280 mμ, and deduct from total absorbance at 280 mμ. Attributing remainder to acetylsalicylic acid, use ratio of acetylsalicylic acid std readings to calc. absorbance due to acetylsalicylic acid at 310 mμ. Deduct this absorbance from total at 310 mμ. Use remainder to calc. quantity of salicylic acid in sample, also to recalc. absorbance due to salicylic acid at 280 mμ. Deduct latter from total absorbance at 280 mμ and use remainder to calc. quantity of acetylsalicylic acid in sample. Alternatively, calc. these two ingredients by simultaneous equations. Amount of acetylsalicylic acid hydrolyzed may be calcd by multiplying amount of salicylic acid by 1.3044.

32.119 Acetophenetidin, Acetylsalicylic Acid, and Salol (Phenyl Salicylate) (*51*)—Official

(**a**) *Acetylsalicylic acid*.—Make detn as soon as possible to prevent any hydrolysis in $NaHCO_3$ soln. Weigh 0.5–1.0 g powd. sample and proceed as in **32.112**(**a**).

(**b**) *Acetophenetidin and salol*.—Evap. combined $CHCl_3$ solns contg the acetophenetidin and salol (corresponding to acetophenetidin and caffeine, **32.112**(**b**) to dryness by means of gentle air blast or fan without heat. Dissolve residue in few ml ether and again evap. to dryness. Treat residue as in **32.125**(**a**), beginning "Add 10 ml 2.5% NaOH soln . . . " For salol, change procedure as follows: Transfer alk. salol soln, freed from acetophenetidin, to vol. flask and dil. to vol. with H_2O. Take aliquot of this soln, contg not >0.08 g salol, for bromination.

32.120 Acetophenetidin, Aminopyrine, and Caffeine (*52*)—Official

(**a**) *Aminopyrine*.—Transfer 2 g powd. mixt. to separator, add 15 ml 10% H_2SO_4 and 50 ml $CHCl_3$, and shake well. Drain $CHCl_3$ into second separator and wash with 15 ml 10% H_2SO_4. Filter $CHCl_3$ into flask. Ext. mixt. in first separator with 5 addnl 25 ml portions $CHCl_3$, washing each portion successively thru the dil. H_2SO_4 as before, filtering, and collecting $CHCl_3$ in flask. Test for complete extn. Reserve this soln for detn of acetophenetidin and caffeine. Add acid washing in second separator to first separator. Make mixt. alk. with NH_4OH (2+3), and remove aminopyrine by successive extns with 25 ml portions $CHCl_3$. Wash each $CHCl_3$ ext. in second separator with 5 ml H_2O contg few drops of NH_4OH, and filter solvent thru cotton into tared beaker. Evap. solvent, add few ml anhyd. ether, and again evap. Dry residue at 80° and weigh as aminopyrine.

(**b**) *Acetophenetidin and caffeine*.—Proceed as in **32.112**(**b**).

Examine residues obtained qualitatively to establish their identity.

32.121 Acetophenetidin, Aminopyrine, Caffeine, and Phenobarbital (*53*)—Official

(**a**) *Aminopyrine*.—Transfer 2.5 g powd. mixt. to separator and proceed as in **32.120**(**a**), reserving $CHCl_3$ soln for detn of acetophenetidin, caffeine, and phenobarbital.

(**b**) *Phenobarbital*.—Evap. the $CHCl_3$ soln of acetophenetidin, phenobarbital, and caffeine to ca 50 ml, transfer soln to separator, and ext. with five 25 ml portions 0.1*N* NaOH. Drain aq. alk. solns and wash each successively with same 5 ml portion $CHCl_3$. Add $CHCl_3$ washings to original $CHCl_3$ soln of caffeine and acetophenetidin. Reserve this soln for further treatment. Acidify aq. alk. soln with HCl (1+3) and shake with successive portions of 25 ml $CHCl_3$-ether mixt. (2+1). Wash each portion of solvent successively with 10 ml H_2O contg few drops of HCl. Evap. solvent, dry residue at 80°, and weigh as phenobarbital.

(**c**) *Acetophenetidin and caffeine*.—Proceed as in **32.112**(**b**).

Acetophenetidin (Phenacetin) and Caffeine (*54*)—Official

32.122 PREPARATION OF SOLUTION

In prepns contg acetophenetidin instead of acetanilid, but otherwise identical, make gross sepn of caffeine-acetophenetidin mixt. as in **32.099**.

32.123 DETERMINATION

(**a**) *Caffeine*.—Treat $CHCl_3$ ext., **32.122**, with 10 ml H_2SO_4 (1+9) and digest on steam bath to ca 5 ml. Dil. with 10 ml H_2O and continue digestion until vol. is again reduced to 5 ml; again add

10 ml H_2O and continue heating to 5 ml. Repeat dilg and evapg until odor of HOAc can no longer be detected in vapors. If, during digestion, particles of acetophenetidin remain on sides of flask, rinse them into soln with few drops of $CHCl_3$.

Cool, transfer with H_2O to separator so that final vol. does not greatly exceed 20 ml, and proceed as in **32.100**(**a**), beginning "Add 50 ml $CHCl_3$ . . . "

Note: Special care must be given to degree of evapn. Should aq. acid soln and suspension of caffeine-acetophenetidin be concd much beyond limits indicated, phenetidin sulfonate is likely to be formed, which later resists acetylation and conversion to acetophenetidin.

(**b**) *Acetophenetidin.*—Wash filter used to dry the $CHCl_3$ with 5 ml H_2O, receiving washings in separator contg soln of phenetidin sulfate. Treat with successive small portions of solid $NaHCO_3$ until, after complete neutralization of free acid, excess of $NaHCO_3$ remains. Add 50 ml $CHCl_3$, and for each 0.1 g acetophenetidin known or believed to have been present, add 5 drops Ac_2O. Shake vigorously, let clear, and drain $CHCl_3$ into second separator contg 5 ml H_2O. Shake this mixt., and after clearing, pass solvent thru small, dry filter into 250 ml erlenmeyer. Repeat extn twice with 50 ml portions $CHCl_3$, washing each portion with the 5 ml H_2O in second separator.

Distill combined $CHCl_3$ exts to ca 10 ml, transfer residual soln with enough fresh solvent to weighed 50 ml beaker or crystg dish, evap. on steam bath to apparent dryness, and finally remove any considerable excess of Ac_2O by repeated addns and evapns of 1 ml $CHCl_3$ and drop of alcohol. (Reformed acetophenetidin should finally appear as whitish, cryst. mass with faint acetous odor that disappears completely on standing several hr in open or over CaO in vac. desiccator.) Weigh at intervals until final wt differs from preceding by not >0.5 mg.

Acetophenetidin (Phenacetin) and Salol (Phenyl Salicylate) (*55*)—Official

32.124 *Acid Hydrolysis Method*

(**a**) *Acetophenetidin.*—Weigh on tared 5.5 cm filter quantity of sample equal to, or multiple of, av. wt of unit dose. Wash with enough successive small portions of $CHCl_3$ to ext. completely all acetophenetidin and salol present in mixt. (ca 40 ml). Collect soln in weighed 100 ml beaker and evap. on warm plate (50–60°) to apparent dryness, using air blast. Let stand 24 hr at room temp. to practically constant wt and weigh.

Transfer cryst. residue with $CHCl_3$ to 50 ml lipped erlenmeyer, evap. solvent with aid of air blast and gentle heat, add 10 ml H_2SO_4 (1+9), and evap. on steam bath until vol. is reduced one-half. Add 10 ml H_2O and continue digestion as before. Add second 10 ml H_2O and evap. to 5 ml. Transfer residue with ca 20 ml H_2O to small separator and ext. with 15, 10, and 5 ml $CHCl_3$, washing each ext. with 5 ml H_2O in second separator. Add wash H_2O in second separator to soln of phenetidin sulfate in first separator and proceed as in **32.123**(**b**), beginning "Treat with successive small portions of solid $NaHCO_3$ . . . "

(**b**) *Salol.*—Subtract wt acetophenetidin from combined wt of the 2 ingredients to obtain wt salol.

32.125 *Alkaline Hydrolysis Method*

(**a**) *Acetophenetidin.*—On small, tared filter or in small beaker weigh quantity of sample contg not >0.08 g salol; exhaust with $CHCl_3$ as in **32.124**(**a**); collect solvent in small lipped erlenmeyer and evap. $CHCl_3$ in air blast without heat. Add 10 ml 2.5% NaOH soln and heat 5 min. on steam bath. Cool quickly to room temp. in running H_2O. Transfer liquid to separator with min. quantity of H_2O and rinse flask with first 20 ml portion $CHCl_3$ to be used in following extn. Ext. alk. soln with three 20 ml portions $CHCl_3$; wash each portion in second separator with 5 ml H_2O, and pass $CHCl_3$ soln thru small, dry filter into 250 ml beaker. Reserve combined alk. soln and washings for detn of salol, (**b**).

Evap. combined $CHCl_3$ exts to ca 5 ml. Transfer with little $CHCl_3$ to small, weighed beaker or crystg dish, evap. on steam bath with air blast, cool, and weigh residual acetophenetidin at intervals to constant wt.

(**b**) *Salol.*—Place reserved combined alk. soln and washings, (**a**), in 500 ml I flask, dil. with H_2O to ca 200 ml, add from buret excess (ca 50 ml) 0.1*N* KBr-$KBrO_3$, **32.128**, add 10 ml HCl, and shake 1 min. and then at intervals during 30 min. Add 10 ml 15% KI soln and shake at intervals during 15 min. Titr. free I with std $Na_2S_2O_3$ soln previously stdzd against the 0.1*N* KBr-$KBrO_3$. 1 ml 0.1*N* KBr-$KBrO_3$ = 0.00178 g salol.

Acetylsalicylic Acid (*56*)—Official

32.126 *Melting Point*

If excipients are present, treat 0.2–0.3 g with small portions of $CHCl_3$ and filter into beaker or evapg dish. Evap. bulk of $CHCl_3$ on steam bath and complete evapn at room temp. Det. m. p. of cryst. residue by USP method.

32.127 *Free Salicylic Acid*

(**a**) *Qualitative test.*—Shake 0.5 g sample in small erlenmeyer with ca 10 ml $CHCl_3$ and filter. Evap., treat residue with 10 ml cold H_2O, and filter. Add 1 drop 10% $FeCl_3$ soln. Violet color indicates free salicylic acid.

(**b**) *Determination.*—In each of 2 colorimeter tubes mix 48 ml H_2O and 1 ml freshly prepd $FeNH_4(SO_4)_2$ soln (1 ml 1*N* HCl and 2 ml 8% $FeNH_4(SO_4)_2.12H_2O$ soln/100 ml). Shake 2.5 g

powd. sample (prepd as in **32.002**) with exactly 25 ml alcohol and filter if necessary. Immediately add 1 ml filtrate to one of colorimeter tubes and 1 ml std salicylic acid soln (0.01 g/100 ml alcohol) to other, and mix. Immediately and rapidly make color comparisons and calc. free salicylic acid on basis of acetylsalicylic acid present. If color is too intense for satisfactory comparison, repeat entire detn, using smaller wt powd. sample.

Total Salicylate

32.128 REAGENT

Potassium bromide-bromate soln.—0.1*N*. Prep. as in **42.018.** Stdze as follows: Transfer 30 ml to I flask, and add 25 ml H_2O, 5 ml 20% KI soln, and 5 ml HCl. Shake thoroly and titr. with 0.1*N* $Na_2S_2O_3$, using starch indicator, **4.004(f)**.

32.129 DETERMINATION

(**a**) *Iodine method.*—Weigh enough sample to yield 0.1–0.2 g acetylsalicylic acid into beaker, add 20 ml H_2O and 1 g Na_2CO_3, and heat on steam bath 15 min. Filter, if necessary, to remove talc. Dil. to 60–75 ml, heat nearly to boiling, slowly add excess (50–80 ml) of ca 0.1*N* I, and proceed as in **32.109(b)**. Multiply wt ppt by 0.4015 to obtain total salicylic acid and deduct free salicylic acid, **32.127.** Remainder$\times$1.304 = wt acetylsalicylic acid.

(**b**) *Bromine method.*—Saponify 0.5 g sample with 10 ml 2% NaOH soln by heating 15 min. on steam bath. Dil. with H_2O in vol. flask to 500 ml. Transfer aliquot of this soln, contg not <0.04 g nor >0.05 g acetylsalicylic acid, to 500 ml I flask, and add 30 ml std KBr-$KBrO_3$ soln. Add 5 ml HCl and immediately insert stopper. Shake frequently during 30 min. and let stand 15 min. Remove stopper just enough to add quickly 5 ml 20% KI soln, taking care that no Br vapors escape, and immediately stopper flask. Shake thoroly, remove stopper, and rinse it and neck of flask with little H_2O so that washings flow into flask. Titr. with 0.1*N* $Na_2S_2O_3$, using starch indicator, **4.004(f)**. 1 ml 0.1*N* KBr-$KBrO_3$ = 0.00230 g salicylic acid, or 0.00300 g acetylsalicylic acid.

32.130 *Combined Acetic Acid (57)*

If excipients are present, weigh accurately 2 g powd. sample and transfer to separator, using ca 25 ml H_2O. Ext. completely with $CHCl_3$, testing last extn by evapg small quantity of the $CHCl_3$ to dryness. (Usually 6 extns with 30, 25, 20, 10, 10, and 5 ml portions $CHCl_3$ are enough.) Filter $CHCl_3$ fractions thru cotton pledget into beaker. Wash original beaker, funnel, and cotton with $CHCl_3$ and add these washings to $CHCl_3$ soln in beaker. Evap. $CHCl_3$ on steam bath and dry residue 15 min. at 80°.

Treat $CHCl_3$ ext., or if no excipients are present, 2 g of the powd. sample, in 150 ml beaker with 30 ml 1*N* NaOH and evap. on steam bath nearly to dryness. Transfer to separator, using 10 ml H_2O, 20 ml 10% H_2SO_4, and finally two 5 ml portions H_2O. Ext. with successive portions of $CHCl_3$, using first portion of 50 ml to rinse beaker used for saponification. Continue extns with $CHCl_3$ until all salicylic acid is removed (ca 6 extns). During these extns keep stopper in separator to guard against loss of HOAc by evapn. Collect $CHCl_3$ fractions in second separator, wash with 25 ml H_2O, and wash this H_2O once with 5 ml $CHCl_3$. Discard $CHCl_3$ exts and return wash H_2O to acid H_2O in first separator.

Transfer acid H_2O contg HOAc and H_2SO_4 to 200 ml vol. flask, wash separators thoroly with H_2O, add to flask, dil. to vol., and mix thoroly. Pipet two 50 ml aliquots, using same pipet and draining same length of time. Place one portion in receptacle suitable for titrn and other in large Pt dish. Titr. first portion at once with 0.5*N* alkali, using phthln. Evap. portion in Pt dish on steam bath to dryness, take up in 10 ml H_2O, and again evap., repeating this process twice more. (During evapn guard against contact with NH_3 vapors.) Take up residue in H_2O and titr. with 0.5*N* alkali, using phthln. Subtract second titrn reading from first and calc. % HOAc in 0.5 g sample. 1 ml 0.5*N* alkali = 0.0300 g HOAc.

Double Titration Method (58)

32.131 PREPARATION OF SOLUTION

(**a**) *Dry extraction method (applicable in all cases).*—Treat weighed quantity of sample contg not <0.3 g acetylsalicylic acid with small portions of $CHCl_3$, filter into beaker, and wash residue with $CHCl_3$ until completely extd. Evap. bulk of $CHCl_3$ on steam bath, finishing with aid of elec. fan without heat.

(**b**) *Wet extraction method (applicable in absence of acids and alkalies, or alkaline earth carbonates).*—Transfer accurately weighed sample to small separator contg ca 20 ml H_2O. Ext. repeatedly with $CHCl_3$, using 30, 25, 20, 15, 10, and 5 ml portions, and test for completeness of extn by evapg portion of final ext. on watch glass. Filter combined $CHCl_3$ portions thru cotton, and wash funnel and cotton with $CHCl_3$. Evap. bulk of $CHCl_3$ on steam bath, finishing with aid of elec. fan without heat.

(**c**) *Acetylsalicylic acid and uncoated tablets containing no excipient.*—Dissolve sample directly in 10 ml neutral alcohol.

32.132 DETERMINATION

Dissolve the dry $CHCl_3$ ext. in 10 ml neutral alcohol, and titr. immediately and rapidly with 0.1*N* alkali, using phthln. Use first persistent pink as end point, since any slight excess of alkali tends to hydrolyze ester quickly. Add vol. of the 0.1*N*

alkali equal to that used in first titrn and then add 5 ml more. Heat on steam bath 15 min. Back-titr. with 0.1N acid. If product is pure, total quantity of alkali consumed will be twice that of first titrn. 1 ml 0.1N alkali consumed in 2 titrns =0.0090 g acetylsalicylic acid.

Acetylsalicylic Acid and Phenobarbital (*59*)—First Action

32.133 APPARATUS

(a) *Spectrophotometer.*—Capable of isolating spectrum of 2 mμ or less in region 230–300 mμ.

(b) *Chromatographic tube.*—Fuse 6 cm length of 5–6 mm tubing to piece of 25 mm tubing ca 25 cm long. (25×200 mm test tube may be used.) Constrict stem slightly ca 2 cm below seal.

(c) *Packing rod.*—Flatten end of glass rod to circular head with clearance of ca 1 mm in tube (b).

32.134 REAGENTS

(a) *Dibasic potassium phosphate soln.*—Approx. 2M. Dissolve 35 g K_2HPO_4 in H_2O, cool to room temp., and dil. to 100 ml.

(b) *Celite No. 545.*—Johns-Manville Corp.

(c) *Washed chloroform.*—Wash USP grade $CHCl_3$ with $\frac{1}{2}$ vol. H_2O in separator.

(d) *Acetylsalicylic acid std soln.*—5 mg/100 ml. Dissolve 100 mg acetylsalicylic acid in $CHCl_3$ and dil. to 100 ml with $CHCl_3$. Dil. 5 ml aliquot to 100 ml with $CHCl_3$.

(e) *Phenobarbital std soln.*—1 mg/100 ml. Dissolve 100 mg phenobarbital in NH_4OH (1+27) and dil. to 500 ml with NH_4OH (1+27). Dil. 5 ml aliquot to 100 ml with NH_4OH (1+27).

32.135 PREPARATION OF SAMPLE SOLUTION

Transfer accurately weighed portion of finely ground tablets contg 60–120 mg phenobarbital to 100 ml vol. flask. Dissolve in $CHCl_3$ by shaking vigorously and dil. to vol. with $CHCl_3$.

32.136 PREPARATION OF CHROMATOGRAPHIC COLUMN

Pack small pledget of glass wool in constricted portion of stem of tube and place pad of glass wool ca 5 mm thick in bottom of large portion of tube. Fasten piece of rubber tubing with attached screw clamp to outlet to limit flow during packing. Clamp tube in vertical position.

To 10 g Celite 545 in mortar add 50 ml $CHCl_3$, and mix with pestle to form slurry. Distribute 10 ml of the 2M K_2HPO_4 soln over surface of slurry and mix thoroly until homogeneous, adding more $CHCl_3$ if necessary. Add this slurry to tube, ca $\frac{1}{5}$ at time, alternately packing and forming flocculent suspension by working packing rod up and down. Celite must be covered with $CHCl_3$ at all times.

After column is packed, remove rubber tube from stem and rinse stem with $CHCl_3$. Check flow rate of column with $CHCl_3$ level ca 5 cm above surface of column. Adjust rate of flow to 2–5 ml/min. by tightening or loosening glass wool pledget in constricted portion of stem. When level of solvent just reaches surface of Celite, place 100 ml vol. flask under stem.

32.137 DETERMINATION OF PHENOBARBITAL

Add 5 ml of the prepd sample soln, **32.135,** from pipet to side of tube near Celite surface. When level of sample soln reaches surface of column, add 5 ml of the washed $CHCl_3$, let sink into column, and repeat with another 5 ml washed $CHCl_3$. After last rinse enters column, add washed $CHCl_3$ to tube and keep level of 4–8 cm $CHCl_3$ above column during elution.

Collect 95 ml eluate in the 100 ml vol. flask. Dil. to vol. with $CHCl_3$, mix, and transfer 20 ml aliquot to 100 ml beaker. Evap. to dryness on steam bath under current of air. Dissolve residue in NH_4OH (1+27) and transfer to 100 ml vol. flask. Rinse, and dil. to vol. with NH_4OH (1+27). Det. absorbance at 240.5 mμ against blank prepd by passing 5 ml $CHCl_3$ thru column as with sample soln.

$$\text{g phenobarbital in sample} = 10A/a,$$

where A is absorbance of soln at 240.5 mμ, and a is absorptivity of phenobarbital at 240.5 mμ obtained by dividing absorbance of std phenobarbital soln in 1 cm cell at 240.5 mμ by its concn (0.01 g/L).

32.138 DETERMINATION OF ACETYLSALICYLIC ACID

Dil. 5 ml original sample soln, **32.135,** to 100 ml with $CHCl_3$ in vol. flask. Dil. 10 ml aliquot of this soln to 100 ml with $CHCl_3$. Det. absorbance of final diln on spectrophotometer at 278 mμ against $CHCl_3$ blank.

$$\text{g acetylsalicylic acid in sample} = 20A'/a',$$

where A' is absorbance of soln at 278 mμ, and a' is absorptivity of acetylsalicylic acid at 278 mμ obtained by dividing absorbance of std acetylsalicylic acid soln in 1 cm cell at 278 mμ by its concn (0.05 g/L).

32.139 Acetylsalicylic acid and Phenolphthalein in Tablets (*60*)—Official

Weigh enough powd. sample, prepd. as in **32.002,** to contain 0.05–0.1 g phthln. Ext. repeatedly with 20 ml portions ether, and filter into separator. Test for complete extn (5–8 extns required).

(a) *Acetylsalicylic acid.*—Shake ether soln, at least 1 min. each time, with two 20 ml portions 4%

$NaHCO_3$ soln (temp. 20° or less). Transfer soln to second separator. Wash ether with two 10 ml portions H_2O and add to $NaHCO_3$ soln. Ext. $NaHCO_3$ soln with 20 ml ether. Drain lower aq. layer into 100 ml vol. flask. Wash ether with small portions of H_2O, rinse into flask, and dil. to mark. Add wash ether to bulk of solvent in original separator. Reserve ether soln for detn of phthln.

Transfer aliquot of the $NaHCO_3$ soln contg not <0.3 g acetylsalicylic acid to separator. (Acid must be sepd from the $NaHCO_3$ soln as rapidly as possible to prevent hydrolysis.) Acidify with HCl (1+3) and ext. liberated acetylsalicylic acid with 30, 20, 20, 10, and 10 ml portions $CHCl_3$-ether (3+2). Wash each ext. with 2 ml H_2O in second separator and filter thru cotton pledget, moistened with the $CHCl_3$-ether mixt., into counterpoised weighed beaker. Test for complete extn. Evap. filtrate to 10–15 ml on H_2O bath, and complete evapn without heat. Dry residue to constant wt at room temp. Wt may be checked by double titrn method, **32.132.**

(b) *Phenolphthalein.*—Ext. original ether soln with 20 ml portions 3% NaOH soln until all phthln is removed (indicated by color). Transfer these alk. exts to second separator, acidify with HCl (1+3), and ext. with $CHCl_3$-ether (3+2). Wash each ext. in third separator with 2 ml H_2O to which has been added 1 or 2 drops HCl (1+3). Filter exts into counterpoised weighed beaker, using cotton pledget moistened with the $CHCl_3$-ether mixt. in stem of funnel. Evap. on H_2O bath and dry to constant wt at 120°. Wt may be checked by tetraiodo method, **32.317.**

Aminopyrine (Pyramidon)—Official

32.140 *Qualitative Tests (61)*

(a) Dissolve 0.01 g sample in 2 ml H_2O and add few drops of yellow HNO_3 (contg HNO_2). Purplish-blue soln is produced.

(b) Dissolve 0.01 g sample in 2 ml H_2O and add 1 ml 10% $FeCl_3$ soln. Purple to violet color develops, which becomes red on addn of H_2SO_4 (1+9).

(c) Dissolve 0.1 g sample in 2 ml H_2O and add few drops of 5% $AgNO_3$ soln. After few sec. purple to violet color is produced, and on standing, deposit of metallic Ag results (useful for detecting aminopyrine in antipyrine).

(d) Dissolve 0.1–0.2 g sample in 2 ml H_2O, add 1 or 2 drops 0.2% $NaNO_2$ soln and few drops of H_2SO_4 (1+9), and shake few sec. Purplish-blue color develops and then gradually disappears, leaving colorless soln. Excess of $NaNO_2$ destroys aminopyrine color. On addn of few more drops of the $NaNO_2$ soln and the dil. H_2SO_4, yellowish-green soln remains after purple disappears if antipyrine is present. (Useful for detecting antipyrine in presence of aminopyrine.)

32.141 *Quantitative Method (62)*

Place 1 g powd. sample in 100 ml vol. flask, add 60 ml 1*N* H_2SO_4, and shake several min. to insure complete soln of the aminopyrine. Dil. to mark with 1*N* H_2SO_4. Filter, if not clear, thru dry filter, rejecting first part of filtrate. Pipet 20 ml aliquot of the soln or filtrate into separator; make distinctly alk. with either NH_4OH or 5% NaOH; and ext. with 20, 15, 10, 10, and 5 ml portions $CHCl_3$. Combine $CHCl_3$ exts in second separator and wash with 2 ml H_2O. Filter $CHCl_3$ soln into weighed beaker thru cotton pledget satd with $CHCl_3$. Ext. wash H_2O with 5 ml $CHCl_3$ and add this to combined $CHCl_3$ exts. Evap. combined $CHCl_3$ exts just to dryness on H_2O bath with aid of elec. fan and dry residue 10 min. at 100°. Cool in desiccator, and weigh as aminopyrine. Identify aminopyrine by its m.p., 106.5–109°, and qual. tests, **32.140,** or microchemical tests, **32.229.**

Antihistamines in Presence of Acetophenetidin, Acetylsalicylic Acid, and Caffeine (APC) (63)—First Action

(Applicable to thonzylamine.HCl, pheniramine maleate, and chlorpheniramine maleate in combination with APC)

32.142 PREPARATION OF STANDARD SOLUTIONS

Prep. sep. std solns of thonzylamine.HCl, pheniramine maleate, and chlorpheniramine maleate by dissolving 250 mg of the antihistamine salt, accurately weighed, in 50.0 ml H_2O. Pipet 5 ml of each soln into 100 ml vol. flasks and dil. to vol. with ca 0.1*N* H_2SO_4. Transfer 10 ml of each acid soln to sep. 100 ml vol. flasks and dil. to vol. with ca 0.1*N* H_2SO_4. (Concn = 2.5 mg/100 ml.) Det. absorbance, A_{std}, of thonzylamine.HCl at 314 mμ, pheniramine maleate at 265 mμ, and chlorpheniramine maleate at 264 mμ.

32.143 DETERMINATION

Place portion of powd. sample contg ca 10 mg of the antihistamine in 125 ml separator. Add 15 ml H_2O and ca 0.5 ml H_2SO_4 (1+1). Ext. with $CHCl_3$, using 30, 20, 20, and 20 ml portions. Re-ext. by passing $CHCl_3$ exts successively thru 2 separators, each contg 10 ml ca 0.1*N* H_2SO_4, shaking vigorously each time. Discard $CHCl_3$ and combine aq. solns.

Make combined solns alk. with 10% NaOH and ext. with 30, 20, 20, and 20 ml portions $CHCl_3$. Again pass $CHCl_3$ exts successively thru 2 separators, each contg 20 ml ca 0.1*N* H_2SO_4, shaking vigorously each time. Discard $CHCl_3$, combine acid aq. solns, and dil. to vol. with ca 0.1*N* H_2SO_4 in 100 ml vol. flask. Transfer 25 ml aliquot to 100 ml vol. flask and dil. to vol. with ca 0.1*N* H_2SO_4. Det. absorbance of soln, A_{sample},

at wavelength of max. absorption against ca $0.1N$ acid as reference blank.

% Antihistamine
$$= (A_{sample} \times 2.5 \times 4 \times 100)/(A_{std} \times \text{mg sample}).$$

Antipyrine and Caffeine (*64*)—Official

32.144 PREPARATION OF SAMPLE

(**a**) Weigh quantity of finely powd. sample equal to, or multiple of, av. unit dose; transfer to filter or beaker and ext. with $CHCl_3$. Evap. $CHCl_3$ on steam bath.

(**b**) With alc. prepns, remove alcohol from measured quantity by heating on steam bath. Ext. residue with three 50 ml portions $CHCl_3$ in separator. Evap. $CHCl_3$ on steam bath.

32.145 DETERMINATION

(**a**) *Antipyrine.*—Transfer residue obtained, **32.144,** which should weigh ca 0.25 g, to 125 ml separator, using two 5 ml portions alcohol-free $CHCl_3$, followed by 10 ml H_2O. (Use of alcohol-free $CHCl_3$ for iodination of antipyrine is necessary to preclude formation of CHI_3, presence of which in composite residue A would vitiate result.) Add 1 g $NaHCO_3$ and 10–15 ml $0.2N$ I (or double quantity of $0.1N$ I), adding latter in small portions and shaking mixt. vigorously after each addn. (I should then be in excess of that required to convert all antipyrine to monoiodo derivative; if it is not, add little more I and shake mixt. again.)

Remove free I with small crystal of $Na_2S_2O_3.5H_2O$ and add 15 ml washed $CHCl_3$, shaking vigorously 1 min. After clearing, drain $CHCl_3$ into second separator; wash with 5 ml H_2O, filter thru small, dry filter into weighed 50 ml beaker, and evap. to apparent dryness on steam bath, using air blast. Repeat extn with 2 (3, if $0.1N$ I has been used) 25 ml portions washed $CHCl_3$, washing, filtering, and evapg each portion as before. Recover any cryst. product sepg around tip of delivery tube, funnel, and edge of filter by judicious washing with $CHCl_3$. Dry nearly colorless cryst. residue of caffeine and iodoantipyrine 30 min. at 100°, cool, and weigh. Designate this weight as A.

Dissolve composite residue in 5 ml HOAc, add 10 ml *satd SO_2 soln*, and wash with hot H_2O into 400–500 ml beaker until final vol. is ca 200 ml. Add enough $AgNO_3$ soln to ppt all the I (ca 0.3 g $AgNO_3$) and few drops of HNO_3, heat nearly to boiling, and stir to agglomerate the AgI. Add 15 ml HNO_3, cover beaker with watch glass, and boil gently 5 min. Decant thru weighed gooch; wash ppt once with little alcohol and then with two 100 ml portions boiling H_2O; and finally transfer AgI to crucible. Wash several times with hot H_2O and again with alcohol to remove traces of org. matter, dry 30 min. at 110°, cool, and weigh. Wt AgI×0.8017 = wt antipyrine.

(**b**) *Caffeine.*—Multiply wt AgI by 1.3374 and subtract product from wt A, in (**a**).

In analysis of mixt. contg caffeine, antipyrine, acetanilid, and Na salicylate, following steps are essential to effect sepn: (1) Extn of caffeine, acetanilid, and antipyrine from aq. alk. soln with $CHCl_3$; (2) hydrolytic treatment with H_2SO_4 of 3 substances thus sepd, preliminary to detn of caffeine and antipyrine as in (**a**).

Cinchophen—Official

In Presence of Salicylates (65)

32.146 REAGENTS

(**a**) *Sodium carbonate soln.*—Dissolve 12.5 g $Na_2CO_3.H_2O$ in enough H_2O to make 100 ml.

(**b**) *Sodium thiosulfate soln.*—$0.02N$. Dil. 200 ml stdzd $0.1N$ $Na_2S_2O_3$, **32.096(a)**, to 1 L with H_2O.

32.147 DETERMINATION

If product is solid, weigh into 50 ml beaker enough finely powd. sample to contain ca 0.15 g cinchophen. Treat with 5, 3, and 3 ml portions of the Na_2CO_3 soln, and filter thru 5 cm paper into 50 ml beaker, finally washing first beaker and paper with little H_2O. Evap. filtrate and washings to complete dryness on steam bath with aid of air blast.

If product is in form of clear soln, transfer measured portion to beaker, and evap. to dryness. In either case, dissolve hot residue in 5 ml HOAc, and transfer to 100 ml vol. flask, using not >10 ml of the acid to complete transfer. Heat to ca 90° on steam bath. Add 25 ml $0.1N$ **I, 32.215,** slowly from pipet with constant agitation of flask, and immediately stopper flask. Let cool, dil. to 100 ml with H_2O, stopper, and let stand 30 min. with occasional thoro agitation. Filter thru small, rapid filter, rejecting first 15 ml filtrate, and immediately titr. 50 ml aliquot with the std $Na_2S_2O_3$ soln, adding starch indicator, **4.004(f)**, as end point approaches. 1 ml $0.1N$ I = 0.0166 g cinchophen, $C_{16}H_{11}O_2N$.

In Presence of Sodium Bicarbonate (66)

32.148 REAGENTS

(**a**) *Solvent.*—Mix 50 ml alcohol and 50 ml ether with 100 ml $CHCl_3$.

(**b**) *Neutral alcohol.*—Neutralize to phthln with $0.1N$ NaOH.

32.149 DETERMINATION

Weigh enough powd. sample to yield 0.3–0.4 g cinchophen, transfer to separator, and add 10 ml 4% NaOH soln to dissolve the cinchophen. Neutralize with HCl (1+3) and add 2 ml excess. Ext. with five 25 ml portions of the solvent, collecting exts in second separator. Wash with 25 ml H_2O and filter exts into beaker. Ext. wash

H_2O with 15 ml solvent and filter into same beaker. Test for complete extn. Evap. solvent to dryness on steam bath. Dissolve residue in 60 ml neutral alcohol. Titr. soln with 0.1*N* NaOH to permanent pink, using phthln. 1 ml 0.1*N* NaOH = 0.02493 g cinchophen.

Salicylic Acid in Presence of Other Phenols (*67*)—Official

32.150 PREPARATION OF SAMPLE

(**a**) *Powders.*—Weigh into vol. flask such quantity of material that aliquot of 25–50 ml contains ca 0.13 g phenol. If acid, make alk. with 4% NaOH, adding 25 ml excess; dil. to mark with H_2O, and shake well.

(**b**) *Liquids.*—Proceed as in **32.151.**

32.151 DETERMINATION

Transfer to separator enough soln to contain ca 0.13 g phenol. Acidify with 10% H_2SO_4 and ext. with ether as in **32.001,** using 20, 15, 15, and 10 ml portions, until extn is complete. Shake combined ether exts with satd $NaHCO_3$ soln, using 15, 15, and 10 ml portions, and finally shake with 15 ml H_2O. Combine $NaHCO_3$ solns and washing, and ext. combined $NaHCO_3$ exts with 15 ml ether. Add latter to main bulk of ether and reserve for phenol detn. Acidify $NaHCO_3$ soln with HCl. Ext. with $CHCl_3$-ether (2+1), using 30, 25, 20, and 10 ml, or until salicylic acid is completely removed. Filter exts into beaker thru cotton previously satd with $CHCl_3$. Evap. to 5 ml on covered steam bath with aid of elec. fan, letting last 5 ml evap. spontaneously. Dissolve residue in 10 ml neutral alcohol and titr. with 0.1*N* NaOH, using phthln. 1 ml 0.1*N* NaOH = 0.01381 g salicylic acid, $C_6H_4OHCOOH$.

HORMONES

Diethylstilbestrol (*68*)—First Action

32.152 REAGENT

Diethylstilbestrol std soln.—Weigh accurately suitable quantity USP Reference Standard Diethylstilbestrol, dissolve in alcohol, and prep. soln contg 20.0 mmg/ml by accurate stepwise diln with alcohol. Prep. working std soln by mixing 25 ml of this soln with 25 ml 1.8% K_2HPO_4 soln.

32.153 APPARATUS

Irradiation containers.—Quartz cells at least 4 ml capacity with clear sides, or 18×150 mm Vycor test tubes, held in rack that does not obstruct effective light beam of cylindrical 15 watt germicidal lamp, may be used conveniently.

32.154 PREPARATION OF ASSAY SOLUTION

(**a**) *Oil solns containing 2 mg or less diethylstilbestrol/ml.*—Using accurately calibrated hypodermic syringe, transfer vol. of sample contg 2 mg diethylstilbestrol to separator contg 50 ml *iso*-octane. Shake mixt. with 10 ml 1*N* NaOH and transfer well-defined aq. layer as completely as possible to second separator contg 50 ml *iso*-octane. Shake vigorously and transfer clear aq. layer to third separator. Repeat extn of the 2 *iso*-octane layers successively with two 10 ml portions 1*N* NaOH, collect aq. layers in third separator, and discard extd *iso*-octane layers.

Acidify combined aq. exts with 3 ml H_2SO_4 (1+1), cool, and ext. diethylstilbestrol with three 30 ml portions $CHCl_3$. Wash $CHCl_3$ exts successively in 2 separators, first contg 20 ml 1% $NaHCO_3$ and second 20 ml H_2O.

Filter washed $CHCl_3$ exts thru cotton pledget moistened with $CHCl_3$ into 100 ml vol. flask, dil. to vol. with $CHCl_3$, and mix.

Transfer 10.0 ml $CHCl_3$ soln, contg 200 mmg diethylstilbestrol, to small erlenmeyer and evap. just to dryness on steam bath with aid of air current. Cool in vac. desiccator 10 min. Add 10.0 ml alcohol, stopper, and dissolve residue by swirling. After 15 min., mix with 10.0 ml 1.8% K_2HPO_4 to prep. assay soln.

(**b**) *Oil solns containing more than 2 mg diethylstilbestrol/ml.*—Dil. convenient accurately measured vol. of oil soln with *iso*-octane to obtain soln contg 0.5 mg diethylstilbestrol/ml. Transfer 4 ml aliquot to separator contg 50 ml *iso*-octane and proceed as in (**a**), beginning "Shake mixt. with 10 ml 1*N* NaOH . . ."

(**c**) *Tablets.*—Transfer accurately weighed portion powd. material contg 2 mg diethylstilbestrol to separator contg 30 ml $CHCl_3$. Add 10 ml H_2O and 1 ml H_2SO_4 (1+1) and shake vigorously. Drain $CHCl_3$ layer into second separator, wash with 5 ml H_2O, and filter thru cotton pledget moistened with $CHCl_3$ into 100 ml vol. flask. Repeat extn with three 20 ml portions $CHCl_3$, dil. combined exts to 100 ml, and mix.

Proceed as in (**a**), fourth par.

32.155 IRRADIATION

(CAUTION: Protect eyes from direct rays of ultraviolet light.)

Test transparency of several irradiation containers as follows: Transfer convenient vols of working std soln to tubes, place them ca 7 cm from 15 watt germicidal lamp, and irradiate soln transversely ca 10 min. Measure absorbances of yellow solns at 418 mμ in suitable spectrophotometer in matched 1 cm cells, against H_2O. Re-irradiate for 1–3 min. intervals, and note irradiation time required for max. absorbance. Repeat irradiation process, varying distance of tubes from lamp, and det. most convenient conditions for developing stable, repeatable colors of max. absorbance (ca 0.7 at 418 mμ).

Transfer portions of working std soln and assay

soln to clean, dry irradiation containers, and irradiate under optimum conditions previously detd. Calc. quantity of diethylstilbestrol in sample.

32.156 TOTAL PHENOLS

Transfer to beaker 20 ml of the $CHCl_3$ ext., **32.154**, contg 400 mmg diethylstilbestrol. Transfer alc. soln contg 400.0 mmg USP Reference Standard diethylstilbestrol to similar beaker, and treat both solns as follows: Evap. to dryness on steam bath with aid of current of air. Dissolve residues in 2.0 ml HOAc with gentle warming. Cool to room temp., add 10 drops H_2SO_4 (1+1), and mix. Cool, add 5 drops 10% $NaNO_2$ soln, and let stand 45 min. with occasional mixing. Wash quantitatively into 25 ml vol. flask with ca 20 ml alc. NH_4OH soln, prepd by mixing equal vols of alcohol and dil. NH_4OH (4+6). Cool in ice bath, and let stand at room temp. 1 hr. Dil. to mark with the alc. NH_4OH soln, and mix. If ppt forms, filter thru dry paper, rejecting first few ml filtrate. Det. absorbances of clear, yellow alk. solns at 420 mμ in tightly stoppered 1 cm cells, in suitable spectrophotometer, against alcohol (1+2). Calc. % total phenols, as diethylstilbestrol, in sample.

Ketosteroids (*69*)—First Action

32.157 APPARATUS

(a) *Spectrophotometer or photometer*.—Capable of isolating spectral band 2 mμ or less at 400–700 mμ. Beckman quartz spectrophotometer fitted with matched 1 cm absorption cells is suitable.

(b) *Separators*.—125 ml, with well-fitting stopcocks lubricated *only with H_2O*.

32.158 REAGENTS

(a) *Benzenesulfonyl chloride*.—Reagent grade, redistd under vac. in all-glass app.

(b) *BQC reagent*.—Prep., just before use, 0.5% soln of cryst. dibromoquinonechloroimide in alcohol. Soln deteriorates progressively, yielding undesirable background colors. Store solid reagent in brown glass bottle in desiccator.

(c) *Buffer soln*.—pH 5.2–5.4. Dissolve 220 g $NaOAc.3H_2O$ (or 133 g anhyd. salt) in 600 ml H_2O. Add 20.0 ml HOAc, dil. to 1 L, and mix.

(d) *Ether*.—USP, freshly washed twice with equal vol. H_2O.

(e) *Girard reagent T* (*trimethylacethydrazide ammonium chloride*).—Recrystallize commercial samples twice from absolute alcohol and dry under vac. at room temp. Recrystd material should be white and practically odorless. Store in tightly stoppered bottle in desiccator.

(f) *Modified iron-Kober reagent*.—Dil. 10 ml stock iron-Kober reagent, **32.165**(a), to 100 ml with H_2SO_4 (2+1) just before use, shaking vigorously to homogeneity.

(g) *Pyridine*.—Redistd over solid KOH in all-glass app.

(h) *Silicon carbide*.—"20 mesh" (Carborundum Co., Niagara Falls, N. Y.).

(i) *Skellysolve C or high-boiling petroleum ether*.

32.159 STANDARD SOLUTIONS

(a) *Estrone std*.—50 mmg/ml. Dissolve 25 mg USP Reference Standard estrone, accurately weighed, in alcohol, and dil. to 500 ml with alcohol.

(b) *Equilin stds*.—50 mmg/ml. Dissolve 25 mg equilin, accurately weighed, in alcohol, and dil. to 500 ml with alcohol. Prep. dild (10 mmg/ml) std in alcohol, required in estrone detn, from aliquot of this soln.

(c) *Equilenin std*.—50 mmg/ml. Dissolve 25 mg equilenin, accurately weighed, in alcohol, and dil. to 500 ml with alcohol.

Stored in tightly stoppered containers in dark, std solns keep for months. Equilin and equilenin available from K&K Labs., Inc., 177-10 93rd Ave., Jamaica 33, N.Y.

32.160 ISOLATION OF KETOSTEROIDS

(a) *Tablets*.—Weigh counted number of tablets and reduce to fine powder without appreciable loss. Weigh accurately sample contg: (1) 5–10 mg ketosteroids; (2) 0.2–0.5 mg estradiol; or (3) 50,000–100,000 International Units estrogens. Transfer to 125 ml separator contg 25 ml H_2O and 2 ml H_2SO_4 (1+1). Ext. with four 20 ml portions $CHCl_3$. Evap. combined $CHCl_3$ exts to ca 5 ml, add 25 ml Skellysolve C, transfer to 125 ml separator with several small portions $CHCl_3$, and proceed as in (c), beginning "Add 10 ml ca 2*N* NaOH . . . "

(b) *Aqueous suspensions*.—Measure portion of sample contg quantity of estrogens specified in (a), and transfer to 125 ml separator. Ext. with six 25 ml portions ether (samples contg polyoxyethylene sorbitol monoöleate, or similar compounds, should be extd with $CHCl_3$, as ether frequently forms emulsions difficult to break). Combine ether exts and evap. to ca 5 ml. Add 25 ml Skellysolve C, transfer to 125 ml separator with aid of several small portions $CHCl_3$, and proceed as in (c), beginning "Add 10 ml ca 2*N* NaOH . . . "

(c) *Oil solns*.—Measure portion of sample contg quantity of estrogens specified in (a), and transfer to 125 ml separator contg 25 ml Skellysolve C. Add 10 ml ca 2*N* NaOH, shake vigorously 2 min., and let layers sep. completely. Transfer aq. layer to second 125 ml separator. Repeat extn with 2 addnl 10 ml portions of the NaOH soln,

adding each ext. to second separator. (Alk. extn should be completed as quickly as possible, because long standing in strongly alk. soln may cause some decomposition of ketosteroids.) Discard Skellysolve soln.

Add H_2SO_4 (1+1) to combined alk. solns until permanent opalescence or ppt forms (acid to litmus). Cool thoroly, add 25 ml washed ether, shake carefully 1 min., and let sep. Transfer acid layer to second 125 ml separator and repeat extn with 25 ml washed ether. Discard acid layer. Ext. ether layers successively with two 5 ml portions 10% Na_2CO_3 soln and two 5 ml portions H_2O. Discard aq. layers. Transfer ether solns to small beaker and carefully evap. to dryness on steam bath in air current, adding few ml alcohol if necessary to aid in removal of residual H_2O.

Dissolve ether ext. in small amount of $CHCl_3$, warming if necessary, and transfer with few ml $CHCl_3$ to 18×150 mm test tube. Carefully evap. $CHCl_3$ on steam bath in current of air. Add 100 mg of the Girard reagent T and 0.5 ml HOAc to test tube, stopper loosely with foil-covered cork, and heat in boiling H_2O bath 5 min.

Cool in ice bath, dissolve reaction mixt. in few ml ice-H_2O, and transfer to 125 ml separator contg ca 25 ml ice-H_2O. Make soln neutral to litmus paper by addn of ca 2*N* NaOH and ext. *at once* with three 15 ml portions $CHCl_3$. Successively drain each portion of $CHCl_3$ into second separator contg 5 ml H_2O, wash, and filter thru cotton pledget wet with $CHCl_3$ into 50 ml vol. flask. Dil. to 50 ml with *alcohol* and retain for estimation of β-estradiol.

Add the 5 ml wash H_2O to aq. soln in first separator. Acidify aq. soln with 2 ml H_2SO_4 (1+1) and let remain 1 hr at room temp. Add 15 ml $CHCl_3$, shake vigorously 1 min., and let sep. Transfer $CHCl_3$ layer to second separator. Repeat extn with three addnl 15 ml portions $CHCl_3$. Wash combined $CHCl_3$ exts with 5 ml H_2O, filter thru cotton pledget wet with $CHCl_3$ into beaker, evap. to small vol., and transfer with $CHCl_3$ to tared 25 ml beaker previously dried in vac. desiccator to constant wt. Carefully evap. to dryness on steam bath in air current, adding few ml alcohol if necessary to aid in removal of residual H_2O. Weigh (residue may be dried in vac. desiccator for semiquant. estimate of ketosteroids). Dissolve residue in enough alcohol for soln to contain 90–120 mmg ketosteroids/ml.

NOTE: It is important that isolation be completed promptly once started. It may be interrupted after obtaining dry residue from ether ext.

32.161 DETERMINATION OF EQUILENIN

Transfer 5 ml aliquot alc. sample soln, **32.160(c)**, to separator. Prep. alcohol blank and stds, one contg 200 mmg equilenin and other 250 mmg equilin, and each dild to 5 ml with alcohol in separators. Add 5 ml buffer soln and 1 ml of the BQC reagent to each separator. Mix, and let stand 2 hr. From buret add 10 ml $CHCl_3$ and mix by careful shaking. Add 20 ml ca 2*N* NaOH and shake vigorously at least 1 min. Drain $CHCl_3$ ext. and filter rapidly thru dry, folded paper. Det. absorbance of sample and std solns at 535 mμ relative to blank. Correct sample reading for equilin content, **32.162**, and calc. quantity of equilenin present.

NOTE: If absorbance obtained from sample is less than that equiv. to 25 mmg equilenin, ketonic residue may be presumed to consist entirely of estrone.

32.162 DETERMINATION OF EQUILIN

Transfer 5 ml aliquot alc. sample soln, **32.160(c)**, to 16×150 mm g-s. test tube. Add chip of SiC, **32.158(h)**, and evap. *just* to dryness by immersing in steam bath. Treat similarly 5 ml alcohol blank and aliquots of the equilin std contg, resp., 100 and 200 mmg equilin. Cool in vac. desiccator 1 hr. Dissolve residue in 2 ml of the dry pyridine, add 0.2 ml benzenesulfonyl chloride, stopper, and let stand overnight.

Mix with 10–15 ml H_2O and wash into separator. Rinse tube with several 10 ml portions H_2O and then with 15 ml $CHCl_3$, and add each to separator. Shake vigorously at least 1 min. and drain $CHCl_3$ layer into 50 ml g-s. erlenmeyer. Repeat extn with 15 ml portion $CHCl_3$, combine exts, and evap. to dryness. Dissolve residue in 2 ml alcohol by gently warming in the stoppered flask, cool, and mix with 4 ml of the buffer soln and 2 ml BQC reagent. Let stand 4 hr (rapid development of pink color indicates incomplete esterification of equilenin).

Add from pipet 5 ml $CHCl_3$ and mix carefully. Add 20 ml ca 2*N* NaOH and shake vigorously at least 1 min. Sep. $CHCl_3$ phase and filter rapidly thru dry, folded paper. Det. absorbances of sample and std solns relative to blank at 570 mμ, and calc. equilin content.

32.163 DETERMINATION OF ESTRONE

Dil. 5 ml alc. sample soln, **32.160(c)**, with 5 ml alcohol. To 1 ml aliquot of this soln in 16×150 mm g-s. test tube add 10 ml of the modified iron-Kober reagent with thoro mixing. Stopper, and mix vigorously. Treat similarly 1 ml alcohol blank, 1 ml aliquot of the estrone std soln, and 1 ml each of the equilenin and the 2 equilin std solns. Immerse loosely stoppered tubes in hot H_2O bath above level of their contents, heat to 75–80°, and keep at that temp. 2 hr.

Cool tubes rapidly in cold H_2O, mix by inverting, and let stand at room temp. 15 min. Det. absorbances of sample and stds relative to blank at 510 mμ (max. for estrone). Calc. and apply

absorbance corrections due to equilin and equilenin, using 10 mmg/ml equilin std for quantities up to 10 mmg equilin in aliquot and 50 mmg/ml std for greater quantities. Calc. estrone content of sample.

Beta-Estradiol (*69*)—First Action

32.164 APPARATUS

(**a**) *Spectrophotometer or photometer.—See* **32.157**(**a**).

(**b**) *Separators.—See* **32.157**(**b**).

(**c**) *Clean, dry, light rubber finger stalls.*—Small or medium size (obtainable from drug supply houses or most drug stores).

(**d**) *Burets.*—Stopcocks lubricated only with reagent. Orifice of one buret should be enlarged, if necessary, to deliver 1 ml Reagent *A* in 30 sec. or less. Protect reagents in burets from moisture with suitable guard tubes.

(**e**) *Chromatographic tube.*—Select 25×200 mm test tube of 3.85–4.00 sq. cm cross-sectional area by measuring height of 50 ml column of H_2O in it. Fuse 6 cm length of 5–6 mm tubing to bottom of tube and slightly constrict this stem ca 2 cm below seal.

(**f**) *Benzene reservoir.*—500 ml separator with 3 mm or larger bore stopcock lubricated only with H_2O. Stem should be ca 10 cm long.

(**g**) *Packing rod.*—Flatten end of glass rod to circular head to provide clearance of ca 1 mm in chromatographic tube.

(**h**) *Leveling rod.*—Sharp-edged rod ca 1.5 cm diam.

32.165 REAGENTS

(**a**) *Reagent A (stock iron-Kober reagent).*—Dissolve 1.054 g $FeSO_4.(NH_4)_2SO_4.6H_2O$ (Mohr salt) in ca 20 ml H_2O; add 1 ml H_2SO_4 and 1 ml 30% H_2O_2. Mix, heat until effervescence ceases, and dil. to exactly 50 ml. To 3 vols of the Fe soln in vol. flask add H_2SO_4, with cooling, to make 100 vols.

Redistill phenol, discarding first 10% and last 5%. Collect distillate with exclusion of moisture in dry, tared g-s. flask of ca twice vol. of the phenol. Place stoppered flask in ice bath to solidify phenol, breaking top crust with glass rod to insure complete crystn. Dry and weigh flask.

Add to phenol 1.13 times its wt of the $Fe\text{-}H_2SO_4$ soln, stopper flask, and let stand without cooling but with occasional mixing until phenol is liquefied (ca 30 min. or less). Shake mixt. vigorously until homogeneous and let stand in dark 16–24 hr. Add to mixt. 23.5% its wt of H_2SO_4 (10+11). Shake mixt. vigorously to homogeneity. Transfer to dry g-s. bottles. Stored in dark and protected from absorption of moisture, this reagent is stable for months.

(**b**) *Reagent B.*—To measured vol. Reagent *A* in g-s. graduated cylinder add 0.45 vol. H_2O, mix, cool, and transfer to dry g-s. bottles. Store in dark and protect from absorption of moisture. Inspect before use and disperse any flocculent ppt by vigorous swirling of reagent. With this precaution, reagent may be used satisfactorily for weeks.

(**c**) *Reagent C.*—To carefully measured vol. Reagent *A* in g-s. graduated cylinder add 0.45 vol. 1*N* HCl, mix vigorously, and place at once in H_2O bath at 25–28°. Use reagent preferably 1 hr and not >3 hr after prepn.

(**d**) *Packing material.*—Celite No. 545 (Johns-Manville diatomaceous earth).

(**e**) *Sodium hydroxide soln.*—0.400*N* (carbonate-free).

(**f**) *Benzene.*—Reagent grade, redistd in all-glass app.

32.166 STANDARD SOLUTIONS

(**a**) *Beta-estradiol std.*—20 mmg/ml. Dissolve exactly 10 mg pure β-estradiol in alcohol and dil. with alcohol to 100 ml in vol. flask. Pipet 10 ml of this soln into 50 ml vol. flask and dil. to vol. with alcohol.

(**b**) *Alpha-estradiol std.*—20 mmg/ml. Prep. as in (**a**) from pure α-estradiol.

(**c**) *Beta-dihydroequilin std.*—10 mmg/ml. Prep. as in (**a**) from 5 mg pure β-dihydroequilin.

Stored in the dark in tightly stoppered containers, std solns keep for months.

32.167 PRELIMINARY DETERMINATION

Apply Methods *A* and *B*, **32.169**, directly to aliquots of $CHCl_3$ soln of diols obtained from **32.160.** (Very turbid solns sometimes resulting from these aliquots should be filtered, along with blank and std solns, thru pledget of fine glass wool packed tightly in lower end of stem of funnel.) Calc. β-estradiol content, disregarding presence of β-dihydroequilin. If wt β-estradiol so detd is equal to 1% or less of wt ketosteroids, report β-estradiol as "Not >1% of ketosteroid content." If β-estradiol content is >1% of wt ketosteroids, repeat detn on aliquot of the $CHCl_3$ soln, using chromatographic sepn, **32.169.**

32.168 PREPARATION OF CHROMATOGRAPHIC COLUMN

Pack fine glass wool into constricted stem of chromatographic tube so that when tube is filled with benzene rate of flow is 2.5–3.0 ml/min. Before packing column, fasten piece of rubber tubing with attached screw-clamp to outlet to control flow during packing.

Cover 1 g Celite in small beaker with benzene. Pipet in 0.5 ml H_2O and mix vigorously with stirring rod until Celite is uniformly wet. With tube ca ½ filled with benzene, place pad of glass wool (ca 1 cm high when gently compressed) at bottom of tube and then transfer Celite mixt. to

tube. Form flocculent suspension by slowly working packing rod up and down as piston thru Celite mixt. Gently compress Celite with packing rod and finish off edges with leveling rod to form level, sharply defined surface on uniform pack ca 1 cm high.

Cover 8 g Celite in mortar with ca 40 ml benzene and distribute over Celite, from pipet, exactly 5 ml 0.400N NaOH. Mix carefully several min. with pestle until Celite appears uniformly wet. Open screw-clamp enough to permit slow drainage during packing of tube. Transfer Celite to tube with spatula in ca 5 portions, suspending each portion and gently packing as above. Finish off top of column, scraping down any Celite on upper wall of tube to form sharply defined, level surface on column of ca 30 ml vol. over initial pack. (Packing too tightly may cause loss of estradiol in forerun, particularly at room temp. much >25°. Celite must be covered with benzene at all times.)

Nearly fill reservoir with benzene and seal stopper with film of H_2O to prevent air leaks. Insert stem of funnel into benzene over the Celite, open stopcock fully, and adjust level of benzene to produce flow rate of 2.0–2.5 ml/min. with screw-clamp fully open. Mark level of benzene on tube, close stopcock, and remove benzene reservoir.

32.169 DETERMINATION

Carefully evap., to just short of dryness, aliquot $CHCl_3$ ext., **32.160**, contg 100–250 mmg total diols calcd as β-estradiol. If necessary, add few ml alcohol near end of evapn to help remove any residual H_2O. Remove last portions of solvent in efficient desiccator connected to vac. 1 hr or more.

Dissolve dry ext. in 5 ml benzene by warming gently; then cool soln to room temp. or below. Remove rubber tubing from partition tube and when benzene *just* stops dropping from tube, transfer diol soln at once to tube, letting it flow down wall near top of Celite (5 ml pipet is convenient for this purpose). When benzene just stops dropping from tube, complete transfer in like manner with 3 addnl 5 ml portions benzene, discarding effluent. Immediately place 50 ml graduated cylinder under tube, add benzene to level marked on tube, and replace benzene reservoir, supporting it at height to maintain that level when stopcock is fully opened. When 30 ml effluent collects in cylinder, replace cylinder as receiver with dry 250 ml beaker previously marked at 170 ml level. (Decrease 30 ml forerun by 2 ml for each 1° that room temp. is >25°.) Collect 170 ml effluent in beaker, conc. soln to 30–40 ml, transfer to 50 ml vol. flask, and dil. to vol.

Det. β-estradiol in soln by Methods A, B, and, if necessary, C.

Method A.—Transfer to dry 18×150 mm test tubes: (1) Aliquot of sample soln contg 10–25 mmg total estradiols; (2) 1 ml α-estradiol std; (3) 1 ml β-estradiol std; and (4) if necessary from Method B, 1 ml β-dihydroequilin std (see NOTE). Add several pieces of SiC, **32.158(h)**, to each tube and rapidly evap. solvent in steam bath (do not use air current) until ebullition from boiling stones just stops. Instantly remove tube, quickly wipe dry, and transfer to efficient vac. desiccator. Keep connected to vac. at least 1 hr.

To each tube and to blank tube add glass bead, and measure 1 ml Reagent A into each tube from buret, quickly wiping outside of tip with piece of absorbent paper before each addn. Immediately cap tubes with rubber finger stalls and let stand 30 min., shaking tubes vigorously at 5 min. intervals. Place in boiling H_2O bath 35 min., removing and shaking each tube few sec. after first 5 min. Transfer to ice bath 2 min.; then remove and add exactly 4 ml H_2SO_4 (7+13) from buret. Let stand 5 min.; then mix by shaking, first gently, then vigorously, to homogeneity.

Measure absorbances of sample and stds relative to blank at 525 mμ (midpoint between max. for α-estradiol and β-estradiol) and at 420 mμ, making any necessary corrections for cell variations.

Mmg total estradiols in aliquot (calcd as β-estradiol)

$$= T_a = 20 \times \frac{A_{525\,m\mu\,(\text{sample})} - \left[(A_{420\,m\mu\,(\text{sample})})/2\right]}{A_{525\,m\mu\,(\beta\text{-estradiol std})} - \left[(A_{420\,m\mu\,(\beta\text{-estradiol std})})/2\right]}$$

Mmg α-estradiol in aliquot (calcd as β-estradiol)

$$= B_a = \text{mmg } \alpha\text{-estradiol in aliquot (from Method } B) \times \frac{A_{525\,m\mu\,(\alpha\text{-estradiol std})} - \left[(A_{420\,m\mu\,(\alpha\text{-estradiol std})})/2\right]}{A_{525\,m\mu\,(\beta\text{-estradiol std})} - \left[(A_{420\,m\mu\,(\beta\text{-estradiol std})})/2\right]}$$

Mmg β-dihydroequilin (DHQ) in aliquot (calcd as β-estradiol)

$$= D_a = \text{mmg DHQ in aliquot (from Method } C) \times \frac{2A_{525\,m\mu\,(\text{DHQ std})} - A_{420\,m\mu\,(\text{DHQ std})}}{A_{525\,m\mu\,(\beta\text{-estradiol std})} - \left[(A_{420\,m\mu\,(\beta\text{-estradiol std})})/2\right]}$$

Mmg β-estradiol in aliquot = T_a − (B_a or D_a or both), depending on composition of aliquot.

NOTE: If β-dihydroequilin in aliquot is >10 mmg, use correspondingly higher std in detg correction for β-dihydroequilin and correct calcn accordingly.

Method B.—Transfer to dry 18×150 mm test tubes: (1) Aliquot of sample soln contg 10–25 mmg total estradiols; (2) 1 ml α-estradiol std; and (3) 1 ml β-dihydroequilin std (see NOTE). Add several pieces of SiC to each tube, evap. solvent, and dry residue as in Method *A*. To each tube and to blank tube, add from buret 1 ml Reagent *B*, quickly wiping outside of tip with piece of absorbent paper before each addn. Immediately cap tubes with rubber finger stalls and place in boiling H_2O bath exactly 2 min., shaking tube after 30 sec. for few sec. without removing from bath. Transfer to ice bath 2 min.; then remove and add from buret exactly 4 ml H_2SO_4 (7+13). Mix by shaking vigorously to homogeneity. Promptly measure absorbances of sample and std relative to blank at 526 mμ (max. for α-estradiol), at 468 mμ (max. for β-dihydroequilin), and at 420 mμ, making any necessary corrections due to cell variation. If

$$A_{468\text{ m}\mu\text{ (sample)}} - \left(A_{526\text{ m}\mu\text{ (sample)}} \times \frac{A_{468\text{ m}\mu\text{ (}\alpha\text{-estradiol std)}}}{A_{526\text{ m}\mu\text{ (}\alpha\text{-estradiol std)}}}\right)$$

does not exceed 20% of $A_{468\text{m}\mu\text{ (}\beta\text{-dihydroequilin std)}}$, then disregarding presence of β-dihydroequilin in like aliquot will result in not >0.8 mmg apparent β-estradiol in Method *A*. This is normally negligible quantity, in which case calc. α-estradiol as follows and omit dihydroequilin std in Method *A*:

$$\text{Mmg } \alpha\text{-estradiol in aliquot} = 20 \times \frac{A_{526\text{ m}\mu\text{ (sample)}} - (A_{420\text{ m}\mu\text{ (sample)}}/2)}{A_{526\text{ m}\mu\text{ (}\alpha\text{-estradiol std)}} - (A_{420\text{ m}\mu\text{ (}\alpha\text{-estradiol std)}}/2)}$$

Otherwise det. β-dihydroequilin by Method *C* and calc. α-estradiol as follows, where DHQ = β dihydroequilin:

$$A_{526\text{ m}\mu\text{ (sample)}}\text{ (corrected)} = A_{526\text{ m}\mu\text{ (sample)}} - (A_{526\text{ m}\mu\text{ (DHQ std)}} \times \text{mmg DHQ in aliquot}/10)$$

$$A_{420\text{ m}\mu\text{ (sample)}}\text{ (corrected)} = A_{420\text{ m}\mu\text{ (sample)}} - (A_{420\text{ m}\mu\text{ (DHQ std)}} \times \text{mmg DHQ in aliquot}/10)$$

$$\text{Mmg } \alpha\text{-estradiol in aliquot} = 20 \times \frac{A_{526\text{ m}\mu\text{ (sample)}}\text{ (corrected)} - (A_{420\text{ m}\mu\text{ (sample)}}\text{ (corrected)}/2)}{A_{526\text{ m}\mu\text{ (}\alpha\text{-estradiol std)}} - (A_{420\text{ m}\mu\text{ (}\alpha\text{-estradiol std)}}/2)}$$

NOTE: If β-dihydroequilin in aliquot is >10 mmg, use correspondingly higher std in detg correction for β-dihydroequilin and correct calcn accordingly.

Method C.—Transfer to 18×150 mm test tubes: (1) Aliquot of sample soln contg up to 20 mmg β-dihydroequilin; and (2) 2 ml β-dihydroequilin std. Add several pieces of SiC to each tube, evap. solvent, and dry residue as in Method *A*. Place tubes in H_2O bath at 25–28° and add rapidly, near bottom of each, 5 ml Reagent *C*. Using long stirring rod, mix residue vigorously with reagent at least 1 min. Leave rod in tube. Measure absorbances of sample and std relative to reagent (also held in bath at 25–28°) at 472 mμ just 30 min. after addn of reagent, and repeat measurement at 5–10 min. intervals until max. absorbance is reached (usually 35–55 min. after mixing).

$$\text{Mmg } \beta\text{-dihydroequilin in aliquot} = 20 \times A_{472\text{ m}\mu\text{ (sample)}}/A_{472\text{ m}\mu\text{ (std)}}.$$

HYPNOTIC DRUGS

32.170 Barbiturates (*70*)—Official

(Applicable in absence of stearic acid)

Weigh 0.3–0.5 g sample into separator, add 10 ml H_2O, and shake well. Add 5 ml 0.5*N* NaOH and shake again. Acidify to litmus paper with HCl (1+3), added dropwise, and add ca 1 ml excess. Ext. with successive 40, 30, 20, 20, and 10 ml portions $CHCl_3$. Test for complete extn by shaking with addnl 10 ml solvent and evapg in sep. beaker.

Combine solvent in second separator and wash with 2 ml H_2O acidified with 1 drop HCl. Filter solvent thru cotton pledget into small weighed beaker. Evap. on steam bath with aid of elec. fan, heat 10 min. at 80–90°, cool in desiccator, and weigh. Add 2 or 3 ml anhyd. ether and evap. solvent. (Usually 2 treatments with 2 ml each of anhyd. ether are enough to remove last traces of $CHCl_3$ and to produce cryst. residue.) Dry at 80–90°, cool, and weigh. Repeat treatment with anhyd. ether and evapn to constant wt. Det. m. p. to check purity of residue.

32.171 *Alternative Method (71)—Official*

(Applicable in presence of stearic acid)

Dissolve residue obtained in **32.170** in 10 ml alcohol, add 20 ml satd $Ba(OH)_2$ soln, and stir well. Filter into separator, and wash residue and filter with two or three 10 ml portions of the $Ba(OH)_2$ soln. Acidify filtrate with HCl (1+3) and proceed as in **32.170,** beginning "Ext. with successive . . . "

Amobarbital Sodium and Secobarbital Sodium (*72*)—Official

32.172 REAGENTS

(a) *Sodium secobarbital.*—Assay by **32.170.**

(b) *Phosphate buffer soln.*—pH 6.85–6.90. Dissolve 6.80 g KH_2PO_4 in ca 500 ml H_2O in 1 L vol. flask. Add 23.6 ml 1.00*N* NaOH soln and dil. to mark with H_2O.

32.173 PREPARATION OF STANDARD CURVE

Accurately weigh ca 100 mg Na secobarbital, transfer to 25 ml vol. flask, dil. to mark with H_2O, and mix well. Pipet 0.5, 1.0, and 2.0 ml aliquots into 50 ml vol. flasks, dil. to mark with buffer soln, and mix. Det. absorbance of each soln at 237 mμ against buffer soln. Plot absorbance against mg barbituric acid/ml calcd as follows:

$$\text{Mg barbituric acid/ml} = \frac{\text{mg Na secobarbital}}{25} \times \frac{\%\ \text{Na secobarbital (from assay)}}{100} \times \frac{128.05}{260.27}.$$

Include 1 or 2 stds with each set of detns.

32.174 DETERMINATION OF TOTAL BARBITURATES

Weigh accurately sample contg ca 400 mg total Na amobarbital and Na secobarbital, transfer to 100 ml vol. flask, and dil. to mark with H_2O. Mix, and let stand 10 min. with occasional shaking. Filter thru quant. paper sufficiently retentive to produce clear soln, discarding first 10–15 ml filtrate. Pipet 2 ml filtrate into 50 ml vol. flask, dil. to mark with buffer soln, and mix well. Det. absorbance at 237 mμ against buffer soln. Det. total barbituric acid in aliquot by reference to std curve and calc. to mg total barbituric acid in sample.

32.175 DETERMINATION OF SODIUM SECOBARBITAL

Pipet 50 ml filtrate into I flask and add 10.0 ml 0.1N KBr-$KBrO_3$, **42.018.** Add 5 ml HCl, stopper at once, and let stand 5 min., shaking occasionally. Add 10 ml 10% KI soln, stopper, and shake. Rinse stopper and neck of flask with H_2O, add starch indicator, and titr. liberated I with 0.1N $Na_2S_2O_3$, **42.035,** using 10 ml buret. 1 ml 0.1N KBr-$KBrO_3$ consumed = 13.02 mg Na secobarbital. Calc. to mg Na secobarbital in sample.

32.176 DETERMINATION OF SODIUM AMOBARBITAL (BY DIFFERENCE)

$$\text{Mg Na amobarbital in sample} = [\text{mg total barbituric acid} - (\text{mg Na secobarbital} \times 0.492)] \times 1.94.$$

32.177 Carbromal (*73*)—Official

Weigh 0.25–0.40 g sample and proceed as in **35.106.** 1 ml 0.05N $Na_2S_2O_3$ = 0.00593 g carbromal.

NOTE: Use <20 ml absorbing soln (15 ml hydrazine sulfate soln and 5 ml 10% NaOH soln) if app. has smaller absorption bulbs than those described in **35.105.**

32.178 Carbromal and Pentobarbital (*73*)—Official

Transfer 0.5–0.7 g sample to separator, and add 15 ml H_2O and 0.5 ml 1N NaOH from pipet. Ext. carbromal with at least five 25 ml portions $CHCl_3$, washing each portion in second separator contg 10 ml H_2O and 2 drops 0.1N NaOH. Filter $CHCl_3$ thru cotton and transfer to tared flask or beaker. Test for complete extn. Evap. $CHCl_3$ soln of carbromal nearly but not quite to dryness on steam bath in air current. Remove container and let stand in air to constant wt.

Combine aq. solns and proceed as in **32.170,** beginning "Acidify to litmus paper . . ." Wt pentobarbital × 1.097 = wt Na pentobarbital in portion taken for assay. Det. m. p. of dried exts. Carbromal melts at 116–119° and pentobarbital at 126–130°.

32.179 Sedormid ® (*74*)—Official

Det. av. wt. of number of tablets and reduce to fine powder. Transfer to small beaker weighed portion of powder, representing ca 0.2 g Sedormid. Add warm $CHCl_3$ and filter thru rapid paper into weighed beaker. Repeat until all Sedormid is extd. Evap. $CHCl_3$ soln on steam bath with aid of elec. fan, removing beaker from bath just before last portions evap., to avoid decrepitation. Add few ml anhyd. ether and evap., taking same precautions as before. Heat 10–15 min. at 100°, cool in desiccator, and weigh. Det. m. p. to check purity of residue (should be 194–197°).

32.180 Sulfonal (Sulfonmethane) or Trional (Sulfonethylmethane) (*75*)—First Action

Mix ca 0.5 g sample with pure, clean sea sand and place mixt. in Knorr tube, **16.017(d),** contg ½″ layer of asbestos. Using bell jar and vac., ext. mixt. with ten 10 ml portions ether, mixing sample with sand with glass rod before each addn of ether. Collect ether exts in tared flask, distill off bulk of ether, and evap. remaining solvent spontaneously, rotating flask to aid evapn. Dry residue in desiccator over H_2SO_4 18 hr and weigh. Identify residue by detn of mixed m. p.

If desired, extn may be made in suitable automatic app., Fig. 64.

INORGANIC DRUGS

Arsenic in Iron-Arsenic Tablets (*76*)—Official

32.181 REAGENT

Std soln of potassium bromate (*or of iodine*).—Stdze against pure As_2O_3. (Concn of this soln is matter of choice. 0.5625 g $KBrO_3$ dissolved in H_2O and dild to 1 L gives soln that is 0.02021N, 1 ml of which = 0.001 g As_2O_3.)

32.182 APPARATUS

Use either Ramberg-Sjöström As flask, Fig. 65A, consisting of 300 ml Kjeldahl flask provided with special outlet tube connected with flask by

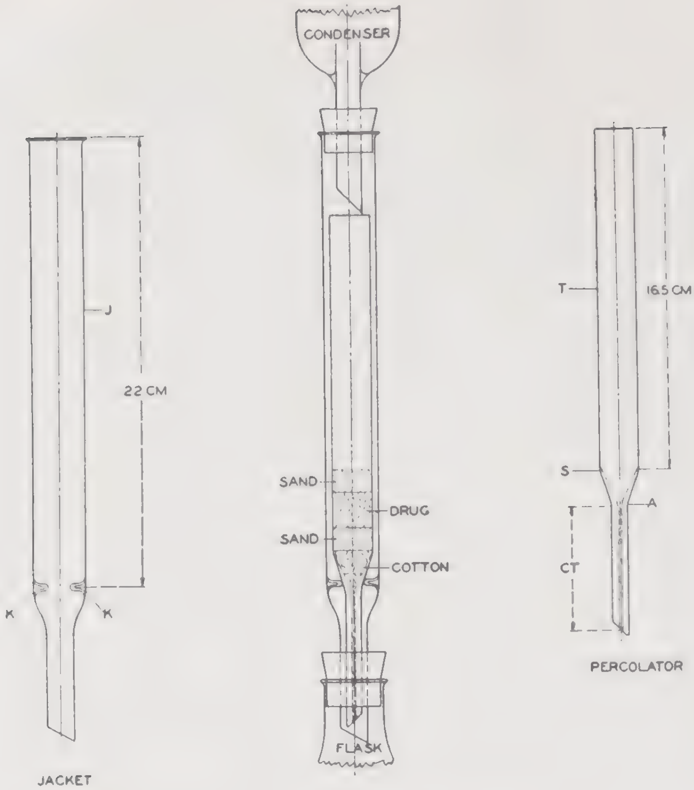

FIG. 64.—AUTOMATIC PERCOLATOR

means of ₸ joint, or 300 ml Kjeldahl flask provided with 13 mm i.d. outlet tube, with constricted tip ca 5 mm, connected with flask by means of rubber stopper, *B*.

32.183 DETERMINATION

Weigh and place in flask 5–10 tablets or pills, add 10–15 ml H_2O, and let soak 30 min. Add, small portions at time, 20 ml fuming HNO_3, cooling if necessary to prevent loss by frothing. When reaction ceases, add carefully and in small portions at time 25–28 ml H_2SO_4. Place flask in inclined position on asbestos mat and heat over small flame. When most of HNO_3 is driven off, and while still heating, drop in 8 ml fuming HNO_3 thru suitably placed thistle tube and heat over larger flame until SO_3 evolves. If after cooling, pptd sulfates are not colorless or pale yellow and are not free from gray or black particles, heat contents of flask further with addnl 10 ml fuming HNO_3. (It is essential that all org. matter be destroyed.)

To cooled mixt. add 30 ml satd $(NH_4)_2C_2O_4$ soln; heat until fumes of SO_3 evolve and, to insure complete destruction of oxalic acid, for 10 min. thereafter over low flame; cool; and add 20 ml

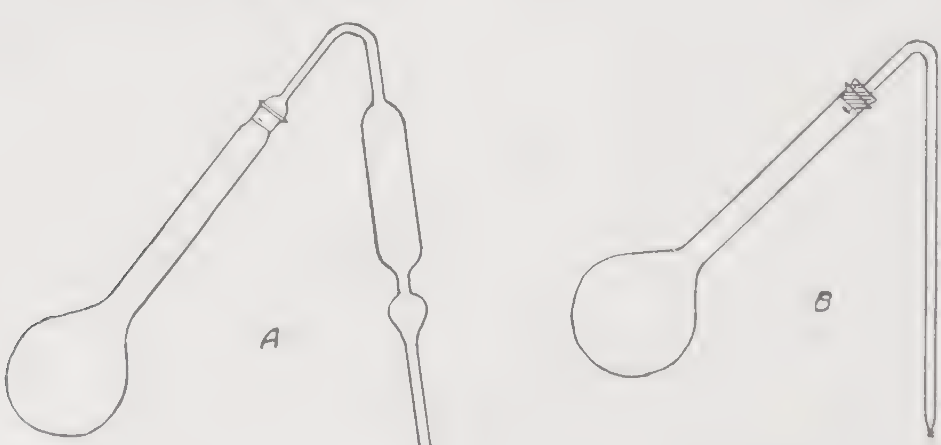

FIG. 65. APPARATUS FOR DETERMINATION OF ARSENIC IN IRON-ARSENIC TABLETS

H_2O while gently swirling flask. Dry neck of flask over small flame and add 30 g NaCl, 5 g $FeSO_4.7H_2O$ (or 1 g $N_2H_4.H_2SO_4$), 1 g NaBr, and 25 ml HCl. Mix contents of flask and connect delivery tube. If Ramberg-Sjöström app. is used, moisten ground-glass joint with 1 drop H_2SO_4. Fix flask in inclined position with tip of outlet tube ca 1 cm under surface of 150 ml H_2O in erlenmeyer surrounded by ice or by cold H_2O.

Distill at such rate that bend at top of tube becomes warm in 4 min. and lower end in ca 8 min. from time heat is applied. Discontinue distn after 10 min., but before removing flame lift distn flask until tip of outlet tube is above H_2O in receiving flask. Let outlet tube drain, remove receiver, and either titr. with the std $KBrO_3$ soln, using 2 drops Me orange (red color of indicator at end point may fade slowly, but color should persist at least 1 min. upon addn of another drop of indicator); or nearly neutralize with NaOH, add 4–5 g $NaHCO_3$, and titr. with std I soln, **4.004(b)**, using starch indicator, **4.004(f)**.

32.184 Arsenic in Iron Methylarsenate (77)—Official

Transfer suitable quantity of sample (0.2 g, if practicable) to Kjeldahl flask. Add 10 g K_2SO_4, 0.3 g starch, and 20 ml H_2SO_4. Digest over low heat until frothing ceases and continue digestion over slightly higher flame until mixt. is colorless. Cool, and add 20 ml H_2O. Dry neck of flask over small flame, cool contents, and add 30 g NaCl, 5 g $FeSO_4.7H_2O$, 1 g NaBr, and 25 ml HCl. Distill as in **32.183.** Conduct blank, using same quantities of reagents.

32.185 Arsenic in Sodium Cacodylate (78)—Official

Transfer 0.2 g sample, accurately weighed, to Kjeldahl flask. Add 10 g K_2SO_4, 0.3 g starch, and 20 ml H_2SO_4. Digest over low flame until frothing ceases. Continue digestion 4 hr or until mixt. is colorless. Cool, dil. with H_2O, and transfer to 500 ml erlenmeyer. Add NaOH soln (1+1) slowly until alk. to litmus paper, and acidify with H_2SO_4. Place flask in H_2O until thoroly cooled, add 5 g $NaHCO_3$, and titr. with 0.1*N* I. Conduct blank, using same quantities of reagents. 1 ml 0.1*N* I = 0.00375 g As, or 0.00800 g anhyd. $Na(CH_3)_2AsO_2$.

Bismuth Compounds

32.186 *Gravimetric Method (79)—Official*
(Lead absent)

Thoroly mix sample and weigh 0.5 g into 500 ml Kjeldahl flask. Ignite gently over small flame, using wire gauze under flask, and increase heat towards end. Let cool, add 15–20 ml HNO_3, evap. to dryness, and ignite as before until yellow or orange Bi_2O_3 is formed. Cool residue and dissolve in 10–15 ml warm HNO_3, using few ml 3% H_2O_2 if residue does not dissolve readily. Boil off excess H_2O_2 and wash into 400 ml beaker with H_2O, rinsing flask well. Dil. to ca 200 ml, make just neutral to litmus with NH_4OH, and add 5 ml HCl. Ppt with H_2S completely.

Transfer ppt to filter paper and wash once with HCl (5+200) and then several times with H_2O. Dissolve ppt of Bi_2S_3 on filter with hot HNO_3 (1+2). Small residue of S (and HgS if Hg salts are present) usually remains. Neutralize filtrate with NH_4OH (2+3) and ppt with 25 ml 20% $(NH_4)_2CO_3$ soln. Conc. to ca 150 ml (by boiling, if desired) and let stand on steam bath 1–2 hr. Collect ppt in previously ignited, weighed gooch, wash with small quantity of H_2O, dry, ignite in muffle at ca 550°, and weigh as Bi_2O_3.

Colorimetric Method—First Action

32.187 APPARATUS

Spectrophotometer or photometer.—Capable of isolating spectral band 2 mμ or less in region of 420–500 mμ. Beckman spectrophotometer fitted with matched 1 cm absorption cells is suitable.

32.188 REAGENTS

(**a**) *Thiourea soln.*—7.5%. Dissolve 15 g thiourea in 200 ml HNO_3 (1+9). Use within 24 hr; filter if necessary.

(**b**) *Bismuth stock soln.*—1 mg/ml. Transfer 124.2 mg Bi subcarbonate, reagent grade, to 100 ml vol. flask, add 5 ml H_2O, and mix. Carefully add 10 ml HNO_3, mix, and heat on steam bath 15 min. Cool, and dil. to 100 ml with H_2O.

(**c**) *Bismuth std soln.*—100 mmg/ml. Dil. 10 ml Bi stock soln (**b**), to 100 ml with HNO_3 (1+9).

32.189 PREPARATION OF STANDARD CURVE

Pipet 1.0, 2.0, 3.0, 4.0, and 5.0 ml Bi std soln (100, 200, 300, 400, and 500 mmg Bi) into 25 ml vol. flasks. Add enough HNO_3 (1+9) to make total vol. of 5 ml in each flask. For blank, pipet 5.0 ml HNO_3 (1+9) into 25 ml vol. flask. Dil. all solns to mark with thiourea soln and mix thoroly. Det. absorbance of each soln at 465 mμ relative to blank, using matched 1 cm cells. Prep. std curve by plotting absorbance against concn Bi in mmg/25 ml.

32.190 PREPARATION OF SAMPLE

Weigh thoroly mixed sample contg 20–80 mg Bi and transfer to 100 ml vol. flask. Add 5 ml H_2O, mix, and slowly add 10 ml HNO_3. Mix, and heat on steam bath 15 min. Cool, and dil. to mark with H_2O. Pipet 10 ml aliquot into 100 ml vol.

flask and dil. to mark with HNO_3 (1+9). Filter if necessary.

32.191 DETERMINATION

Pipet 5 ml sample soln into 25 ml vol. flask and dil. to mark with thiourea soln. Det. absorbance at 465 mμ relative to blank prepd by dilg 5.0 ml sample soln to 25 ml with HNO_3 (1+9). Det. Bi in sample aliquot from std curve. Bi in sample = Bi in aliquot × 200.

32.192 Calcium Gluconate (*80*)—Official

(Applicable to prepns whose aq. solns are neutral and which do not contain salts of other optically active hydroxy acids.)

Weigh two 0.5 g portions Ca gluconate or two 1 g portions powd. tablets contg 50% or less of the salt. If chocolate or fatty base is present, wash samples several times on hardened filter with absolute ether and warm residue until ether is driven off.

Transfer each portion to sep. 25 ml vol. flask, add 15 ml H_2O, and warm until Ca salt dissolves. (Samples contg cocoa will have undissolved residue.) Cool mixt. to room temp.

To one flask (No. 1) add 3.5 g *finely pulverized uranyl acetate*, stopper, and shake in machine 1 hr. (If agitation is not vigorous enough, >1 hr of shaking may be required.) Let other flask (No. 2) stand. If sample contains chocolate, add little alumina cream, **29.021(b)**, to each flask. Cool to 20°, dil. contents of flask No. 1 to vol. with *uranyl acetate soln* (10 g shaken with 95 ml H_2O until satd and then filtered), and flask No. 2 with H_2O. Filter, and polarize each soln in 200 mm tube, using 50 mm tube contg 1.8% $K_2Cr_2O_7$ soln as light filter. If soln is too dark to read in 200 mm tube, make reading in 100 mm tube and multiply result by 2. If A = rotation in °S of Soln No. 2 and B = rotation of Soln No. 1, with 1 g sample % $Ca(C_6H_{11}O_7)_2 = 4.34 \times (B-A)$, and with 0.5 g sample % $Ca(C_6H_{11}O_7)_2 = 8.52 \times (B-A)$.

32.193 Calcium, Phosphorus, and Iron in Vitamin Preparations (*81*)—Official

Transfer representative portion of well-mixed sample contg at least 10 mg P, 50 mg Ca, and 1 mg Fe to 100 ml Pt or porcelain dish. Ash at temp. not >525° until apparently C-free (gray to brown). Cool, moisten with 20 ml H_2O, break up ash with stirring rod, and add 10 ml HCl cautiously under watch glass. Rinse off watch glass into dish and evap. to dryness on steam bath. Add 50 ml HCl (1+9), heat on steam bath 15 min., and filter thru quant. paper into 200 ml vol. flask. Wash filter and dish thoroly with hot H_2O, cool filtrate, dil. to mark, and mix.

(a) *Phosphorus.*—Using aliquot contg 2–5 mg P, proceed as in **20.030**.

(b) *Calcium.*—Transfer aliquot contg 20–40 mg Ca to suitable beaker, dil. to 100 ml, and proceed as in **6.011**. Correct for $KMnO_4$ consumed in blank detn.

(c) *Iron.*—Transfer aliquot contg 0.2–0.5 mg Fe to 100 ml vol. flask, add enough HCl (1+9) to yield 2 ml concd acid, and dil. to vol. Proceed as in **13.013(a)**, beginning "Pipet 10 ml aliquot into 25 ml vol. flask . . . " Det. Fe in sample by comparison with stds prepd as in **13.012.**

Effervescent Potassium Bromide with Caffeine (*82*)—Official

32.194 PREPARATION OF SAMPLE

Powder sample, transfer immediately to dry bottle, and seal tightly. Thoroly mix powder in bottle by rotating and shaking before removal of sample for analysis. Weigh all needed portions as nearly at same time as possible. Avoid extreme temps and humidities when opening and storing samples.

32.195 DETERMINATIONS

(a) *Potassium bromide.*—Weigh 2.5–3 g sample and transfer to 250 ml erlenmeyer. Add 50 ml H_2O and swirl gently, avoiding loss of soln by spattering. Acidify soln with HNO_3 and then add 5 ml excess. Add 30 ml 0.1*N* $AgNO_3$, **42.025, 42.029,** and 2 ml ferric indicator, **32.239(b)**. Let mixt. stand several min. and swirl occasionally to aid in flocculating the AgBr. Titr. excess of 0.1*N* $AgNO_3$ with NH_4CNS soln, **32.239(a)**. 1 ml 0.1*N* $AgNO_3$ = 0.0119 g KBr.

(b) *Caffeine.*—Weigh 12–15 g sample, transfer to separator, and slowly add 50 ml H_2O, avoiding loss of soln by spattering. If soln is not alk. to litmus, make alk. with 5% NaOH soln. Add 50 ml $CHCl_3$, shake vigorously, and filter into beaker. Repeat extn with two 50 ml portions $CHCl_3$. Wash filter and funnel with few ml $CHCl_3$ to remove any adhering caffeine. Evap. combined $CHCl_3$ filtrates on H_2O bath to ca 10 ml, finally transferring residual liquid to small weighed beaker. Let soln evap. by gentle heat and air blast. Dry residue to constant wt at 80° and weigh.

Elixir of Five Bromides (*83*)—Official

32.196 PREPARATION OF DILUTION

Transfer 50 ml sample to 1 L vol. flask, dil. to mark, and mix. Measure aliquots of this diln at original temp. of sample.

32.197 DETERMINATIONS

(a) *Ammonium bromide.*—Place 200 ml aliquot of the diln in Kjeldahl flask; add small piece of

paraffin and excess of 10% NaOH soln (ca 5 ml). Distill the NH_3 into excess of std acid (40 ml 0.1*N* usually is enough). Titr. excess acid with 0.1*N* NaOH, using Me red, **32.023**(**b**). 1 ml 0.1*N* acid = 0.00980 g NH_4Br.

(**b**) *Calcium bromide.*—Pipet 100 ml aliquot of the diln into casserole or Pt dish and evap. to dryness. Ignite at dull red (ca 525°) until org. matter is thoroly charred. Cool, add 5 ml HCl (1+3) to dissolve Ca salts, filter, and wash well with hot H_2O. Return filter and unoxidized C to casserole or dish and ignite at moderate temp. until residue is white. Treat residue with 5 ml HCl (1+3), filter, and wash with hot H_2O, combining filtrates.

Det. Ca as in **6.011,** and reserve filtrate for detn of Na, K, and Li. If 0.1*N* $KMnO_4$ is used, 1 ml = 0.0100 g $CaBr_2$.

(**c**) *Lithium bromide.*—Dil. filtrate and washings from Ca detn to 200 ml and mix. Evap. 100 ml aliquot to dryness and drive off all NH_4 salts by heating to faint red (ca 525°) in Pt dish. Treat residue with little H_2O, filter into Pt dish, add few ml HCl, and evap. to dryness.

Complete conversion of alkali bromides to chlorides by treating residue with Cl-H_2O and evapg to dryness. Repeat addn and evapn of Cl-H_2O twice more, or until there is no apparent darkening of soln due to liberation of Br. Proceed as in **31.028** and **31.029,** beginning "Dissolve mixed chlorides in hot H_2O, filter, and wash." (Since Na and K are to be detd directly, it is not necessary to weigh mixed chlorides.) $Li_2SO_4 \times 1.5800$ = LiBr.

(**d**) *Sodium bromide.*—Remove combined KCl and NaCl from gooch by washing with hot H_2O, dil. to 50 ml, and use 5 ml aliquot for detn of Na. Proceed as in **6.024,** beginning "add 100 ml of the Mg uranyl acetate soln . . . " Calc. to NaBr, using factor 0.0688.

(**e**) *Potassium bromide.*—Use 25 ml aliquot of the soln of KCl and NaCl and proceed as in **31.029,** fourth par., beginning "Add enough Pt soln, **2.059**(**b**), to convert KCl and NaCl to K_2PtCl_6 and Na_2PtCl_6, and evap. to dryness." Calc. to KBr, using factor 0.4897.

(**f**) *Total bromine.*—Transfer 20 ml of the diln to 500 ml flask. Add 100 ml H_2O, 2 ml HNO_3, and excess of 0.1*N* $AgNO_3$ (usually 30 ml). Titr. excess $AgNO_3$ with 0.1*N* NH_4CNS, using Fe alum indicator. 1 ml 0.1*N* $AgNO_3$ = 0.00799 g Br.

Elixir of Three Bromides (*83*)—Official

32.198 PREPARATION OF DILUTION

Dil. 25 ml elixir to 250 ml. Measure aliquot of this diln at original temp. of sample.

32.199 DETERMINATION

(**a**) *Ammonium bromide.*—Transfer 50 ml aliquot to Kjeldahl flask provided with trap and condenser; add 150 ml H_2O and excess of 10% NaOH soln (ca 5 ml). Distill the NH_3 into excess of 0.1*N* H_2SO_4 (ca 50 ml), and titr. excess acid with 0.1*N* NaOH, using Me red, **32.023**(**b**). 1 ml 0.1*N* H_2SO_4 = 0.00980 g NH_4Br.

(**b**) *Potassium bromide.*—Evap. 10 ml aliquot and ignite at dull red (ca 525°). Treat residue with hot H_2O, filter, and wash into porcelain evapg dish. Convert bromides to chlorides by treating residue with 2 portions of Cl-H_2O, evapg between addns, and proceed as in **6.020,** beginning "acidify with few drops HCl . . . " $K_2PtCl_6 \times 0.4897$ = KBr.

(**c**) *Sodium bromide.*—Transfer 5 ml aliquot to suitable beaker and proceed as in **6.024,** beginning "add 100 ml of the Mg uranyl acetate soln . . . " Wt Na-Mg uranyl acetate × 0.0688 = NaBr.

(**d**) *Total bromine.*—Transfer 10 ml aliquot to flask and add slowly and with agitation 30 ml 0.1*N* $AgNO_3$, 2 ml HNO_3, and 2 ml $FeNH_4(SO_4)_2$ soln. Titr. excess $AgNO_3$ with 0.1*N* NH_4CNS. 1 ml 0.1*N* $AgNO_3$ = 0.00799 g Br.

Hypophosphites in Sirups (*84*)—Official

(Applicable in absence of phosphates; if phosphates are present, make suitable correction)

32.200 *Method I.*

(**a**) *Total hypophosphites.*—Pipet 25 ml sample into 100 ml vol. flask, dil. to mark, and mix thoroly. Pipet 10 ml aliquot into suitable flask. Add 25 ml HNO_3 and boil on hot plate to 2–3 ml; add 10 ml HNO_3 and boil again to 2–3 ml. Cool, and add 20 ml H_2O. Add NH_4OH in slight excess and barely dissolve ppt formed with few drops HNO_3, stirring vigorously. To hot soln add 70 ml molybdate soln, **2.017**(**a**), for each 0.1 g P_2O_5 present. Digest 1 hr at ca 65°, and test for complete pptn by addn of more reagent to clear supernatant. Filter, and wash with NH_4NO_3 soln, **2.017**(**b**).

Dissolve ppt on filter with NH_4OH (1+1) and hot H_2O, and wash into beaker to vol. of not >100 ml. Nearly neutralize with HCl, using litmus paper as indicator. Cool, and from buret add slowly (ca 1 drop/sec., stirring vigorously) 15 ml magnesia mixt., **2.017**(**c**), for each 0.1 g P_2O_5 present. After 15 min. add 12 ml NH_4OH and let stand overnight. Filter, and wash ppt with dil. NH_4OH, **2.017**(**d**), until washings are practically Cl-free. Dry; ignite first at low temp. and finally to constant wt, preferably in elec. furnace, at 950–1000°. Cool, and weigh as $Mg_2P_2O_7$. $Mg_2P_2O_7 \times 0.6377 = P_2O_5$.

(**b**) *Calcium.*—Using prepd soln (**a**), first sentence, pipet 20 ml aliquot into 400 ml beaker and dil. to 100 ml. Add 2 ml HCl, 15 ml 10% NH_4OAc

soln, and slight excess of satd $(NH_4)_2C_2O_4$ soln. Heat to boiling and let ppt settle at temp. just below boiling. Filter hot, wash with 1% NH_4OAc soln, dry, moisten with H_2SO_4, ignite gently, and weigh residue as $CaSO_4$. $CaSO_4 \times 0.2944 = Ca$.

Method II.

(Not applicable in presence of other reducing agents or of phenolic compounds)

32.201 DETERMINATION

Transfer 50 ml sample, measured in 50 ml vol. flask, quantitatively to 250 ml vol. flask, dil. to mark with H_2O, and mix well. (This procedure is followed in case of the sirup of $NH_4H_2PO_2$; for sirups contg larger quantities of hypophosphites, dil. to 500 ml in vol. flask.)

Transfer 50 ml aliquot to 250 ml vol. flask, dil. to mark with H_2O, and mix well. Of this soln, transfer 50 ml aliquot (equiv. to 2 ml sirup) to g-s. 250 ml flask; add 50 ml KBr-$KBrO_3$ soln, **32.128,** and 20 ml 10% H_2SO_4; stopper, shake well, and let stand 2 hr. Add 10 ml 20% KI soln, shake flask, and titr. liberated I with $0.1N$ $Na_2S_2O_3$ soln, **32.096(a)**, to straw color; add 2 ml starch soln, **4.004(f)**, and titr. until colorless. Conduct blank detn in same way. 1 ml $0.1N$ $Na_2S_2O_3 = 0.00165$ g H_3PO_2; 1 ml $0.1N$ $Na_2S_2O_3 = 0.00208$ g $NH_4H_2PO_2$.

32.202 Iodine (*85*)—**Official**

Transfer quantity of sample contg not >0.1 g of the iodide (0.05 g is ample) to crucible, preferably Ni. If sample contains only slight quantity of org. material, add 1 g starch. Add 2–3 g solid KOH. If sample is solid, add 10–15 ml alcohol before adding the KOH. Alkali must be thoroly mixed with sample to prevent loss of I in muffle (accomplished by stirring, leaving stirring rod in crucible, or by heating and swirling on steam bath until the KOH is in soln). Dry and char thoroly. (Use as low temp. as possible to prevent loss of I, in no event more than dull red.) Ext. charred mass with hot H_2O, filter into erlenmeyer, and wash well with hot H_2O.

Neutralize filtrate with H_2SO_4 (1+1), make alk. again with 4% NaOH soln, and add 1 ml excess. Heat to boiling and add satd $KMnO_4$ soln slowly until $KMnO_4$ color remains after several min. of boiling. Then add ca 0.5 ml excess, continue boiling ca 5 min., and let cool. Add enough $KMnO_4$ to completely oxidize all iodide to iodate so that $KMnO_4$ color, not brown MnO_2 color, is present at end of boiling period. Add few ml alcohol and place on steam bath. ($KMnO_4$ color should be bleached; if it is not, add little more alcohol.) When ppt has settled, filter, and wash with hot 1% NH_4Cl soln. If filtrate is not clear, digest on steam bath until the MnO_2 can be retained on filter. After cooling, add 1–2 g KI, acidify with HCl, and titr. with $0.1N$ $Na_2S_2O_3$. 1 ml $0.1N$ $Na_2S_2O_3 = 0.00277$ g KI, 0.00250 g NaI, or 0.00212 g I.

32.203 Iodine Ointment (*86*)—**Official**

(a) *Free iodine.*—Weigh (to 1 mg) ca 2 g ointment, and transfer to 250 ml I flask. Melt on H_2O bath (not >70°), add 30 ml $CHCl_3$, mix well, and then add 30 ml H_2O. (All of base should be dissolved in the $CHCl_3$ before H_2O is added.) Titr. with $0.1N$ $Na_2S_2O_3$, using starch indicator, **4.004(f)**. Approach end point dropwise, shaking flask vigorously to insure that all I has been extd from $CHCl_3$ layer. 1 ml $0.1N$ $Na_2S_2O_3 = 0.01269$ g I.

(b) *Potassium iodide.*—Pour liquids from free I detn (a), into 500 ml I flask, rinsing flask with 200 ml H_2O, added in several portions. (It is desirable to maintain this vol. within rather narrow limits.) Add 0.5 ml *0.2% alc. p-ethoxychrysoidin indicator* and 1–4 drops $0.1N$ NaOH (to neutralize). (Aq. layer should now be clear yellow.) Titr. with $0.1N$ $AgNO_3$, approaching end point dropwise and swirling frequently. ($AgNO_3$ soln causes turbidity due to formation of colloidal AgI and development of reddish-brown color similar to that observed in over-titrd Volhard detn. End point, which is produced by 1 drop of the $AgNO_3$ soln, is characterized by flocculation of the colloidal AgI and complete disappearance of reddish-brown tinge, leaving almost clear, pale yellow supernatant.) Ml $0.1N$ $AgNO_3$ − ml $0.1N$ $Na_2S_2O_3$ (a) = ml consumed by iodide originally present. 1 ml $0.1N$ $AgNO_3 = 0.0166$ g KI.

Nitrites in Tablets—Official

Hydrazine Method (*87*)

(Applicable in presence or absence of nitrates or chlorides)

32.204 REAGENT

Hydrazine sulfate soln.—$0.1N$. Dissolve ca 3.25 g $N_2H_4.H_2SO_4$ in H_2O and dil. to 1 L. Stdze against $0.1N$ I, **42.016,** as in detn.

32.205 DETERMINATION

Weigh 20 tablets, reduce to fine powder, and mix well. Weigh accurately sample contg ca 130 mg $NaNO_2$, place in 100 ml vol. flask, dil. to vol. with H_2O, and mix well. Filter, discarding first few ml filtrate. Pipet 50 ml aliquot filtrate and 50 ml of the $N_2H_4.H_2SO_4$ soln into 300 ml erlenmeyer, add 5 ml H_2SO_4 (3+47), wash flask down with H_2O, mix (soln should be acid), and let stand 30 min. with occasional swirling. Add $NaHCO_3$ in small quantities, while tipping flask, until reaction ceases. Titr. excess $N_2H_4.H_2SO_4$ with $0.1N$ I to end point which remains 1 min.

Starch indicator may be used in titrn of colored solns. 1 ml $0.1N$ $N_2H_4.H_2SO_4$ = 2.300 mg $NaNO_2$.

Silver Proteinates (*88*)—Official

32.206 *Total Silver*

Place 1 g sample, accurately weighed, in 500 ml Kjeldahl flask; add 15 ml H_2SO_4 and then 10 ml HNO_3. Place on steam bath few min., with occasional rotation, to insure homogeneous mixt., and boil to white fumes. Add more HNO_3, boil again to clear colorless soln, and cool. Add 100 ml H_2O and boil until free of N oxides. Cool, dil. to 300 ml, add 5 ml HNO_3 and 5 ml $FeNH_4(SO_4)_2$ soln, **32.239(b)**, and titr. with $0.1N$ NH_4CNS. 1 ml $0.1N$ NH_4CNS = 0.01079 g Ag.

32.207 *Ionizable Silver Compounds*

Weigh strip of commercial dialyzing tubing 55 mm wide and ca 1 foot long, wet with H_2O until uniformly pliable, shake free of adhering H_2O, and partially dry by rolling in clean paper towel. Reweigh while still moist and place in 250 ml beaker. (Sheets of dialyzing parchment paper may be used in place of tubing. Over one end of glass tube 10 cm long and ca 2.5 cm o.d., fold and secure with rubber band square piece of parchment paper in form of sack large enough to hold sample soln. Dialyzing material should be kept in humid container to prevent breaking when handled.)

Weigh 1 g sample, dissolve in 15 ml H_2O, and transfer to dialyzing tube. Calc., and add enough H_2O to beaker to make 100 ml (this insures 20 ml in dialyzing tube and 80 ml in beaker). Adjust tubing to form "U" in beaker, cover with watch glass, and keep cool and in dark 24 hr.

(a) *Qualitative test.*—Test few ml of clear, colorless soln from beaker for Ag ions by addn of few drops of HCl (1+3) and trace of HNO_3.

(b) *Determination.*—If Ag ions are present, remove 50 ml of the clear, colorless soln (representing 0.5 g sample) from beaker, dil. to 100 ml, and add 2 ml $FeNH_4(SO_4)_2$ soln, **32.239(b)**, and 2 ml colorless HNO_3. Titr. with $0.01N$ NH_4CNS and calc. to % by wt ionizable Ag. 1 ml $0.01N$ NH_4CNS = 0.001079 g Ag.

MERCURIAL DRUGS

Mercury (*89*)—First Action

(Applicable to Hg in phenylmercuric chloride, HgI_2, nitromersol, HgO ointment, and calomel tablets.)

32.208 REAGENTS

(a) *Strychnine sulfate soln.*—Approx. $0.01M$; 4.3 g/500 ml.

(b) *Valser's reagent.*—Dissolve 10 g KI in H_2O and dil. to 100 ml. Sat. with HgI_2 (ca 14 g) and filter.

32.209 APPARATUS

(a) *Digestion flask.*—Round bottom or acetylation; 100 ml capacity fitted to H_2O-cooled straight tube condenser with ₸ joint.

(b) *Gooch crucibles.*—Fitted with 21 mm filter paper disks, covered with thin layer of asbestos, and dried at 105°. Use to filter and weigh ppt of strychnine.HI.HgI_2.

32.210 PREPARATION OF SAMPLES

Weigh (avoid use of metal containers) or measure accurately quantity of sample contg 20–100 mg Hg (optimal ca 50 mg) and treat as follows:

(a) *Solns of organic mercurials.*—Transfer sample to beaker and evap. just to dryness with low heat (60–70°) and current of air. Dissolve residue in ca 5 ml 10% NaOH soln and transfer to digestion flask. Rinse beaker with four 3–4 ml portions H_2O and add rinsings to digestion flask. Add excess liquid Br to soln and connect flask to condenser. Boil 4–5 min. and add 3 ml HCl thru top of condenser. Continue to heat soln until Br collects in condenser tube. Remove heat and cool until Br returns to soln in digestion flask.

Alternately heat and cool until Br has almost completely dissipated. (After 3 intervals of heating, flow of H_2O thru condenser may be discontinued to aid in removing Br.) Let flask cool, and rinse inside of condenser with ca 5 ml H_2O. Disconnect flask and rinse tip of condenser with small stream of H_2O from wash bottle. Filter thru 9 cm paper into 150 ml beaker, and rinse flask and filter with four 5 ml portions H_2O.

(b) *Ointments.*—Transfer sample to digestion flask and add 5 ml HCl (1+3) followed by 5 ml satd Br-H_2O. Place small pieces of porcelain, SiC, or few glass beads in flask to prevent bumping. Connect flask to condenser and fit flask over hole cut in asbestos board so that bottom of flask extends just below under surface of board. Heat over low flame, maintaining slow and continuous ebullition ca 10 min., and then cool to room temp. Disconnect flask and decant aq. portion thru 9 cm paper into 150 ml beaker. Take precautions to retain all ointment base in flask. Rinse neck of flask into filter with few drops of H_2O from wash bottle. Add 1 ml HCl (1+3), 1 ml satd Br-H_2O, and 8 ml H_2O to flask and reflux. Again cool contents of flask and decant aq. phase thru filter.

Repeat refluxing and decanting with two 10 ml portions H_2O and finally rinse condenser tube into flask with ca 5 ml H_2O. Disconnect flask, rinse condenser tip, and decant rinsings thru filter. Rinse filter with 2 small portions H_2O from wash bottle.

Test for complete removal of Hg by adding 5

ml H_2O and 2 drops HCl (1+3) to digestion flask and refluxing as before. Pass this soln thru original filter into 50 ml beaker. To filtrate add 1 drop of 10% KI soln and 1 drop of the strychnine sulfate soln. No turbidity should be produced. If extn is incomplete, repeat refluxings with H_2O until all Hg is removed. Reserve all test solns showing presence of Hg to add to major portion after pptn of Hg.

(**c**) *Calomel tablets.*—Weigh at least 20 tablets and det. av. wt. Grind to fine powder and transfer accurately weighed portion to digestion flask. Add 10 ml satd Br-H_2O and 5 ml HCl (1+3). Connect flask to reflux condenser and boil contents gently until most of Br vapors collect in condenser. Discontinue heating until Br returns to soln in flask. Repeat alternate heating and cooling until Br vapors are dissipated. Cool flask and contents to room temp. and rinse condenser tube with ca 10 ml H_2O. Disconnect flask and rinse condenser tip into flask. Filter soln thru gooch into 150 ml beaker. Rinse flask with three 5 ml portions H_2O and pass rinsings thru crucible, and finally rinse crucible with fine stream of H_2O.

(**d**) *Tablets containing purgative drugs.*—If tablets contain purgative drugs, add 10 ml alcohol to weighed sample in flask. Heat on steam bath with gentle agitation until alcohol begins to boil. Remove flask, cool under tap, and filter supernatant thru gooch fitted with asbestos mat. Retain as much of insol. residue in flask as possible. Rinse flask and contents with three 10 ml portions alcohol and two 5 ml portions H_2O, and decant thru crucible as above. Remove asbestos mat with fine wire or needle and transfer to flask. Rinse crucible with 10 ml satd Br-H_2O and 5 ml HCl (1+3), and add rinsings to flask. Connect flask to condenser, and treat as in (**c**).

32.211 DETERMINATION

Add 10 ml 10% KI soln to filtrate, and if necessary, evap. on steam bath under current of air to ca 50 ml. If soln has not previously been acidified, add 3 ml HCl (1+3). Add 1% $NaHSO_3$ soln until I color is discharged, and keep soln free from I color by addn of the $NaHSO_3$ soln until final filtration is made. Add strychnine sulfate soln slowly from buret or pipet until ppt coagulates and settles rapidly. (Strychnine sulfate soln may be added as rapidly as it will flow from buret if theoretical quantity is used, based on 1 ml soln for each 4 mg Hg expected to be present.) Avoid undue excess of strychnine because of slight solubility of its hydriodide.

Let ppt settle and test for complete pptn by adding 2–3 drops of the strychnine sulfate soln to clear supernatant. If pptn is incomplete, indicated by cloudiness around the drops, add strychnine sulfate soln in 1 ml increments until pptn is complete. Let ppt remain in beaker with occasional stirring 0.5–1 hr.

Decant supernatant thru weighed gooch, **32.209**(**b**). Wash ppt into crucible with fine stream of H_2O. Completely transfer ppt to crucible, and wash residue and crucible with three 5 ml portions H_2O. Scrub beaker thoroly with policeman. Transfer crucible and holder to another small suction flask and wash residue with 2–3 ml H_2O. Test filtrate for complete removal of strychnine by addn of Valser's reagent. If necessary, continue washing ppt with small portions of H_2O until last washings give no more than faint opalescence upon addn of Valser's reagent. Always test main filtrate by addn of ca 1 ml of the strychnine sulfate soln to assure complete pptn of Hg. If pptn was incomplete, repeat detn. Dry crucible 1 hr at 105°, cool in desiccator, and weigh. Calc. % Hg compound in sample on basis of mol. wt of 916.78 for ppt of strychnine $.HI.HgI_2$.

Mercurochrome (Merbromin) (*90*)

32.212 *Tests for Purity—Procedure*

(**a**) Acidify portion of mercurochrome soln with 10% H_2SO_4 and filter off ppt. Filtrate is colored only slightly yellow.

(**b**) Pass H_2S into portion of filtrate. No ppt or coloring occurs.

(**c**) Add few ml 10% HNO_3 to another portion of filtrate and add $AgNO_3$ soln. No ppt forms.

32.213 *Total Solids in Solution—Official*

Pipet 10 ml mercurochrome soln into tared, extra-wide-form weighing bottle and evap. to dryness on steam bath. Let dry overnight in open bottle in desiccator contg H_2SO_4. Weigh.

32.214 *Determination of Mercury—Official*

Pipet 10 ml ca 2% mercurochrome soln into 500 ml tall beaker and evap. to dryness on steam bath (or weigh accurately ca 0.2 g of the powder). Dissolve residue in 4 ml H_2O and add slowly, with constant mixing, 10 ml H_2SO_4. Incline beaker and add cautiously small portions of finely pulverized $KMnO_4$, mixing after each addn, until deep purple color shows considerable excess has been added. Let stand 30 min., mixing occasionally. Mixt. should still be purple.

Add 100 ml H_2O and mix thoroly. Add small portions of finely pulverized oxalic acid, mixing after each addn, until soln is clear. Filter thru small filter into 400 ml beaker, wash original beaker and filter until filtrate measures ca 200 ml,

and pass H_2S thru soln 20 min. Warm on steam bath until ppt of HgS settles quickly after stirring, and again pass H_2S thru warm soln 5 min. Filter soln immediately into weighed gooch; wash ppt on filter well with H_2O, 3 times with alcohol, and then with 4 or 5 portions of CCl_4 or CS_2, letting liquid run thru crucible without suction; finally wash with ether. Dry ppt to constant wt at 100° and weigh as HgS. HgS $\times$ 0.8622 = Hg.

Test dried ppt qualitatively for Hg and other heavy metals. If slow filtration occurs during washing with H_2O, let ppt drain, and wash once with alcohol; then continue as directed.

Mercurous Chloride (Calomel) in Ointments (*91*)—Official

32.215 REAGENTS

Iodine std soln.—0.1*N*. Dissolve ca 14 g I in soln contg 18 g KI in 100 ml H_2O and dil. to 1 L. Stdze this soln against std $Na_2S_2O_3$ soln, **32.096(a)**.

32.216 DETERMINATION

Weigh accurately ca 1 g ointment, transfer to 250 ml g-s. erlenmeyer and treat with ca 50 ml $CHCl_3$. When base is dissolved, decant thru dry, closely packed asbestos mat in Caldwell crucible, using light suction. Wash flask and contents several times with 20–30 ml portions $CHCl_3$, decanting thru crucible. Let any residual $CHCl_3$ in flask evap. and transfer asbestos mat and contents to flask, wiping sides of crucible and mouth of flask with damp piece of filter paper and adding it to flask. Add 2.5 g KI and 50 ml 0.1*N* I, stopper, and mix well. Let flask stand ca 1.5 hr or until soln of calomel is complete, agitating frequently and fairly vigorously. Titr. with 0.1*N* $Na_2S_2O_3$, **32.096(a)**, adding 1 or 2 ml excess and using starch indicator, **4.004(f)**. When all traces of I disappear, back-titr. with the std I soln to blue color. 1 ml 0.1*N* I = 0.02361 g Hg_2Cl_2.

32.217 Mercurous Chloride (Calomel) in Tablets (*92*)—Official

Count and weigh representative number of tablets. Pulverize quantity of tablets and weigh accurately enough well-mixed sample to contain 0.19–0.26 g (3–4 grains) HgCl. Transfer to 200 ml g-s. erlenmeyer, add ca 50 ml H_2O, acidify with HOAc, and after sol. fillers dissolve, decant with aid of suction thru tightly packed asbestos mat placed on plate of Caldwell crucible. Wash once with H_2O by decantation and then successively with alcohol and ether. Transfer removable plate holding mat and insol. material to original flask, washing into flask any insol. material adhering to sides of crucible. Add 2.5 g KI, 10 ml H_2O, and then 30 ml std I soln, **32.215**. Complete detn as in **32.216**.

32.218 Mercurous Iodide in Tablets (*93*)—Official

Weigh accurately enough well-mixed powd. sample to contain 0.19–0.26 g (3–4 grains) HgI. Transfer sample to 200 ml g-s. flask, and proceed as in **32.217**, omitting addn of H_2O after the KI. 1 ml 0.1*N* I = 0.03275 g HgI.

Note. Some commercial tablets are difficult to filter thru asbestos mat without loss of HgI. Placing few drops of alumina cream, **29.021(b)**, on mat before filtration is started (wash free from NH_3), satisfactorily prevents loss, tho it retards filtration.

32.219 Mercury in Mercurial Ointment (*94*)—Official

After mixing ointment thoroly with glass rod, avoiding contact with metals, weigh 1 g sample into erlenmeyer. Add 20 ml H_2O and 20 ml HNO_3, and heat gently over small flame until red fumes cease to evolve. Cool, and decant aq. soln from the ointment base into separator. Wash ointment base with 50 ml boiling H_2O, cool, and decant into separator. Repeat washing until all Hg is removed.

Shake combined solns in separator with 50 ml ether. Transfer aq. soln to erlenmeyer. Wash ether soln with three 10 ml portions H_2O until Hg is removed, adding washings to flask. Add 3 ml $FeNH_4(SO_4)_2$ soln, **32.239(b)**, and titr. with 0.1*N* NH_4CNS. 1 ml 0.1*N* NH_4CNS = 0.01003 g Hg.

32.220 Mercury in Ointment of Mercuric Nitrate (*95*)—Official

Transfer, to 200–300 ml erlenmeyer, 3–5 g sample, accurately weighed, using glass or bone spatula. Add 40 ml HNO_3 (1+1) and few glass beads, and insert short-stem funnel into neck of flask. Boil gently 1–1.5 hr on hot plate or over low flame. (With latter, use piece of asbestos with circular hole under asbestos wire gauze.) Add 30 ml H_2O, using part to wash funnel. Cool enough (ca 20° or below) to cause solidification of unconsumed fat. Filter thru 11 cm paper into 200 ml vol. flask. Wash fat, flask, and filter, using ca 100 ml 1% HNO_3. Dil. to vol. and mix well. Reserve fat to test for complete extn as below.

Test for complete extn of Hg from fat and its removal from filter, etc., by repeating HNO_3 digestion ca 30 min. on residual fat in flask or on filter, completing this as separate detn, including $KMnO_4$ digestion. Add any titrn in excess of 1–2 drops (0.05–0.08 ml 0.1*N* NH_4CNS) resulting from this test portion to that obtained by titrg main ext.

Transfer 100 ml aliquot to 500 ml erlenmeyer. Add 7 ml HNO_3, 5 ml H_2SO_4, and 2 g powd.

$KMnO_4$, and rotate to dissolve. Heat just to boiling over low flame or on hot plate. Boil gently 45 min., maintaining excess of $KMnO_4$, indicated by dark purple color. (Excess is essential.) When adding $KMnO_4$ to boiling liquid, use smaller portions (ca 0.5 g or less) to avoid loss due to frothing.

CAUTION: Use of greater excess of $KMnO_4$ than necessary is not objectionable, but proportionately more H_2O_2 is required to remove it and the MnO_2 at end of digestion. Usually ca 10 g is required. Rate of consumption and total $KMnO_4$ consumed appear to vary with temp., org. matter present, and period of heating. Large quantity of MnO_2 formed may lead to wrong conclusion concerning color indicative of excess of $KMnO_4$. Frequent examination of soln is necessary. Observation of this color is aided by looking thru supernatant toward white background while holding container in inclined position.

Remove excess $KMnO_4$ and dissolve MnO_2 by adding H_2O_2 (5–10% prepd from 30%) dropwise to hot soln. When colorless, add 2% $KMnO_4$ soln slowly until faint pink or brown persists ca 1 min. If large quantity of MnO_2 forms at this point, again use H_2O_2 sparingly; then use $KMnO_4$ to discharge the H_2O_2. Discharge color from last addn of $KMnO_4$, including weak brown color from MnO_2, by adding dropwise just enough 8% $FeSO_4.7H_2O$ soln. Cool to ca 20°, add 3 ml ca 0.5*N* $FeNH_4(SO_4)_2.12H_2O$, and titr. with the std NH_4CNS. 1 ml 0.1*N* $NH_4CNS = 0.01003$ g Hg.

MICROSCOPIC TESTS

Microchemical Tests

For Alkaloids and Related Amines—Official

32.221 REAGENTS

(a) *Ammoniacal silver nitrate soln.*—Mix 2.5 ml 4% $AgNO_3$ soln with 2.5 ml NH_4OH (1+5). Prep. fresh as needed.

(b) *Ammonium hydroxide soln.*—10% NH_3 (2+3).

(c) *Ammonium thiocyanate soln.*—Dissolve 5 g NH_4CNS in 100 ml H_2O.

(d) *Bismuth iodide soln.*—(1) Prep. stock concd $Bi(NO_3)_3$ soln by dissolving 50 g Bi subnitrate in 70 ml HNO_3 (1+1) and dilg to 100 ml with H_2O. (2) Dissolve 1.25 g KI in 4.5 ml H_2O and add 0.5 ml stock concd $Bi(NO_3)_3$ soln. Prep. fresh when soln darkens appreciably.

(e) *Bismuth iodide in diluted sulfuric acid soln.*—Dissolve 1.25 g KI in 2.0 ml H_2O, and add 2.5 ml H_2SO_4 (1+3) and 0.5 ml stock concd $Bi(NO_3)_3$ soln, (d)(1). Prep. fresh daily.

(f) *Disodium phosphate soln.*—Dissolve 5 g $Na_2HPO_4.12H_2O$ in 100 ml H_2O.

(g) *Gold bromide in hydrochloric acid soln.*—Dissolve 1 g $HAuCl_4.3H_2O$ and 1.5 ml 40% HBr in 18 ml HCl. (Satd aq. NaBr soln may be substituted for the HBr.)

(h) *Gold chloride soln.*—Dissolve 1 g $HAuCl_4.3H_2O$ in 20 ml H_2O.

(i) *Hydrochloric acid.*—5% (1+7).

(j) *Iodine potassium iodide soln.*—Dissolve 1.27 g I and 2 g KI in 5 ml H_2O and dil. to 100 ml.

(k) *Lead iodide soln.*—To aq. KOAc soln (1+3) add 1 drop Me red and HOAc until yellow changes to orange; then, while gently warming, sat. with PbI_2, cool, and filter.

(l) *Mercuric chloride soln.*—Dissolve 5 g $HgCl_2$ in 100 ml H_2O.

(m) *Mercuric chloride-sodium chloride soln.*—Dissolve 5 g $HgCl_2$ and 0.75 g NaCl in 100 ml H_2O.

(n) *Platinic chloride soln.*—Dissolve 5 g $H_2PtCl_6.6H_2O$ in 100 ml H_2O.

(o) *Potassium cadmium iodide soln.*—Dissolve 3 g CdI_2 in 18 ml H_2O contg 6 g KI.

(p) *Potassium ferrocyanide soln.*—Dissolve 5 g $K_4Fe(CN)_6.3H_2O$ in 100 ml H_2O.

(q) *Potassium hydroxide soln.*—Dissolve 5 g KOH in 100 ml H_2O.

(r) *Potassium iodide soln.*—Dissolve 5 g KI in 100 ml H_2O.

(s) *Potassium permanganate soln.*—Dissolve 1 g $KMnO_4$ in 100 ml H_2O.

(t) *Reinecke salt soln.*—Dissolve 0.1 g $NH_4[Cr(NH_3)_2(CNS)_4].H_2O$ and 0.03 g $NH_2OH.HCl$ in 10 ml alcohol. Filter, and store in refrigerator. (Reagent is stable 6 months or more.)

(u) *Sodium benzoate soln.*—Dissolve 5 g Na benzoate in 100 ml H_2O.

(v) *Sodium carbonate soln.*—Dissolve 5 g $Na_2CO_3.H_2O$ in 100 ml H_2O.

(w) *Sodium iodide soln.*—Dissolve 5 g NaI in 100 ml H_2O.

(x) *Sodium nitroprusside.*—$Na_2Fe(CN)_5NO.2H_2O$ crystals.

(y) *Zinc chloride soln.*—Dissolve 5 g $ZnCl_2$ in 100 ml H_2O.

(z) *Zinc potassium iodide soln.*—Dissolve 5 g $Zn(OAc)_2.3H_2O$ and 20 g KI in 100 ml H_2O.

32.222 PREPARATION OF SAMPLES

(a) *Usual controls.*—Dissolve 0.4 or 0.2 mg of the pure alkaloid salt in 0.04 ml H_2O to make ca 1:100 or 1:200 soln.

(b) *Alkaloids in compounds.*—Sep. alkaloid in pure form by extg it from ammoniacal soln with suitable immiscible solvent, and evap. solvent. Dissolve little of residue in min. of 0.1*N* HCl and dil. with H_2O, if necessary, to ca alkaloid concn specified in (a) or in test.

(c) *Hypodermic tablets.*—Dissolve portion of tablet in drop of H_2O to ca same alkaloid concn specified in (a) or in test.

32.223 IDENTIFICATION

Place drop (ca 0.04 ml) of alkaloid soln on clean glass slide, add drop of reagent, and without stirring or covering, examine under microscope, using magnification of ca 100–150×. Note kind of crystals formed and compare their characteristics with descriptions given, **32.224**, and also with a control. Use polarizing microscope if available, and note characteristics such as birefringence and dichroism.

32.224 *Characteristics of Microchemical Tests for Alkaloids and Related Amines*

ALKALOID	REAGENT	DESCRIPTION OF CRYSTALS
Aconitine (*96*)	Sodium carbonate	In 1:3000 soln heated to 50° in test tube. Small, transparent, hexagonal plates; also rods in contact.
Apomorphine (*97*)	Potassium iodide	1:50. Small crystals that have sharp, clear-cut angles like those of diamond.
	Gold chloride	Red-brown, fine needles, in dense masses in all solns to 1:10,000.
	Hydrochloric acid	1:50. Small rods singly and in clusters.
Arecoline (*96*)	Bismuth iodide	Red, rhombic crystals.
Atropine (*98*)	Iodine potassium iodide	Small, dark rods and triangular plates form in great numbers, singly and in groups.
Benzyl morphine (*101*) (Peronin)	Potassium iodide	1:200. Dense rosettes of needles. Crystals are formed readily in dil. solns (1:1000) in form of sheaves of needles.
	Ammonium thiocyanate	1:200. Rosettes and sheaves of needles in acid or neutral soln.
	Hydrochloric acid	1:100. Rods, usually notched at ends and often in rosettes, are formed on stirring.
Berberine (*102*)	Hydrochloric acid	Satd soln; fine yellow needles. (Avoid excess reagent.)
Brucine (*103*)	Potassium iodide	Long masses of transparent, rectangular plates also rosettes of thin plates.
	Mercuric chloride	Small, dense rosettes.
Choline (*104*)	Reinecke salt	Add 1 drop acetone to 1 drop H_2O soln of base. Stir, add 1 drop reagent, and stir again. 1:100. Thin, hexagonal plates and star-shaped forms. 1:1000–1:10,000. Six-sided, more coffin-shaped plates; sometimes rosette aggregates of plates on edge, resembling needles.
	Platinic chloride and sodium iodide	1:100 in H_2O. Add 1 drop H_2PtCl_6 soln, stir, and add small drop NaI soln without stirring. Small black rectangular prisms and slender black rods.
Cinchonidine (*105*)	Sodium benzoate	Rosettes and sheaves of needles spreading to large size
	Platinic chloride	Rosettes of transparent plates.
	Sodium carbonate	Spherical crystals, but not needles as in cinchonine.
Cinchonine (*105*)	Sodium carbonate	Dark rosettes, composed of radiating needles, form immediately.
	Disodium phosphate	Similar to crystals formed by Na_2CO_3, but more burr-shaped.
Cocaine (*106*)	Platinic chloride	Delicate, feathery crystals, later becoming heavier in structure.
Codeine (*106*)	Potassium cadmium iodide	Silvery, circular masses, crystg into dark rosettes of irregular outline.
	Iodine potassium iodide	Heavy, red-brown ppt; crystallizes very slowly in yellow blades extending in branches (never red).

32.224 *Characteristics of Microchemical Tests for Alkaloids and Related Amines*—Continued

ALKALOID	REAGENT	DESCRIPTION OF CRYSTALS
Cotarnine (*102*)	Platinic chloride Mercuric chloride Potassium ferrocyanide	1:200. Hair-like crystals, yellow and curving. Colorless, long, branching needles. Acidify with 1 drop 5% HCl; globules that develop into dense, burr-shaped crystals; also amber-brown plates.
Dihydromorphinone (Dilaudid ®) (*107*)	Sodium nitroprusside	To minute quantity (<1 mg) in 2 drops H_2O add minute fragment of reagent. Elongated 6-sided prisms; also in aggregates.
Ephedrine (*108*)	Bismuth iodide in dild sulfuric acid	1:200. Long, brownish-orange, radiating and interlacing needles and branching rods.
Ethylhydrocupreine (*120*) (Optochin ®)	Ammonium thiocyanate	1:100 in 0.1*N* HCl. Long, straight needles.
Ethylmorphine (*101*) (Dionin ®)	Iodine potassium iodide Mercuric chloride	1:200. Groups of yellow needles, branching later. Transparent plates, often with notched ends; singly and in groups. Stir to start crystn.
Heroin (*109*) (Diacetylmorphine)	Platinic chloride	Spherical clusters of golden yellow needles form slowly around nucleus; cluster disintegrates on standing.
Homatropine (*110*)	Gold chloride	1:200. Green-gold blades, often with pointed ends and united in pairs; surfaces appear etched on long standing.
Hydrastine (*97*)	1 drop 5% HCl and 1 drop potassium ferrocyanide	1:100. Spheres of radiating crystals. Shake slide to start crystn. Avoid excess reagent.
Hydrastinine (*101*)	Potassium permanganate Mercuric chloride 1 drop 5% HCl and 1 drop potassium ferrocyanide	1:500. Immediate red plates, often with serrated edges. In concd soln, great number of large red or brown plates with deeply cut edges. 1:500. Transparent needles forming branches rapidly in neutral and acidified solns. 1:200. Yellow rhombic plates and tree-like crystals.
Hyoscyamine (*110*)	Gold chloride	Thin, transparent, nearly colorless irregular plates, often curved. Crystals form slowly in 1:100 to 1:200 soln. Shaking slide aids crystn.
Morphine (*106*)	Potassium cadmium iodide Iodine potassium iodide	Silvery, gelatinous ppt, crystg in dense masses of fine needles. Small drop of reagent produces heavy, red-brown ppt, slowly crystg in shining, red, overlapping plates extending in branches.
Narceine (*102*)	Iodine potassium iodide, or zinc potassium iodide Platinic chloride	1:400. Blue, radiating needles, sometimes with yellow dichroism. Beautiful feathery rosettes develop in all solns.
Narcotine (*102*)	Potassium hydroxide or ammonium hydroxide	1:200. White, amorphous ppt that crystallizes slowly; dense rosettes of needles.
Nicotine (*111*)	Mercuric chloride Mercuric chloride-sodium chloride	Radiating, transparent blades form in presence of slight excess of H_2SO_4; feather-like blades form in presence of HCl. Radiating, transparent blades.
Papaverine (*112*)	Zinc chloride	Thin, rectangular plates in excess HCl.
Physostigmine (*113*)	Lead iodide Gold bromide in HCl	1:100. Radiating, serrated plates. 1 mg in 1 drop H_2O. Brown, dendritic aggregates.

32.224 *Characteristics of Microchemical Tests for Alkaloids and Related Amines*—Continued

ALKALOID	REAGENT	DESCRIPTION OF CRYSTALS
Pilocarpine (*98*)	Platinic chloride	Crystals form slowly; layers of thin, yellow, triangular plates of delicate structure.
Procaine (*112*)	Platinic chloride Gold chloride and HCl	Spherical crystals of radiating branches. Irregular, radiating branches.
Quinidine (*105*)	Potassium iodide	Small, triangular crystals in great numbers; best in 1:1000 diln; sol. in excess reagent.
Quinine (*105*)	Disodium phosphate	Silvery, sheaf-like crystals.
Racephedrine (*116*) (*dl*-Ephedrine)	Bismuth iodide in dild sulfuric acid	1:200. Large orange plates and red prisms and grains.
Scopolamine (*110*) (Hyoscine)	Gold chloride	Clusters of pale yellow, transparent blades, with coarse, saw-toothed edges form immediately on shaking slide. Crystals grow to large size in 1:200 soln.
Sparteine (*111*)	Gold chloride	Large numbers of blade-like crystals varying in size according to concn.
Stovaine (*114*) (Amylocaine)	1 drop HCl and 1 drop gold chloride	1:50. Dendritic crystals.
Strychnine (*115*)	Platinic chloride Potassium cadmium iodide	Crystals form immediately in clusters and singly in small, wedge-shaped needles that move about field. Silvery masses, slowly forming rosettes.
Yohimbine (*96*)	Sodium carbonate	In 1:1000 soln heated to 50°. Fine needles in sheaf-like bundles and rosettes.

For Sympathomimetics (116)—First Action

32.225 REAGENTS

(a) *Bismuth iodide in diluted sulfuric acid soln.*—*See* **32.221(e)**.

(b) *Gold chloride in diluted phosphoric acid soln.*—Dissolve 1 g $HAuCl_4.3H_2O$ in 20 ml H_3PO_4 (1+2).

(c) *Platinic chloride in diluted phosphoric acid soln.*—Dissolve 1 g $H_2PtCl_6.6H_2O$ in 20 ml H_3PO_4 (1+3).

(d) *Sodium tetraphenylboron soln.*—Aq. soln (1+20).

32.226 IDENTIFICATION

(a) *Direct test.*—Add drop of reagent to little of powd. solid or crushed tablet and spread out on slide with little stirring. Do not stir to homogeneity as local concns and dilns will assist crystn. Let stand to evap. to higher acid concn if necessary for crystal formation.

(b) *Volatility test.*—Place small amount of substance or crushed tablet in depression of cavity slide, add drop 5% NaOH soln, and stir briefly. Place very small drop of reagent on thin slide, invert over cavity slide, and let stand. As crystals appear, examine with inverted slide in place. After observing crystals or after 1 hr or more exposure, if only few or no crystals form, reinvert thin slide with hanging drop, and let stand for gradual evapn of H_2O from reagent drop. Examine for crystals.

32.227 *Characteristics of Microchemical Tests for Sympathomimetics*

SYMPATHOMIMETIC	REAGENT	TEST	DESCRIPTION OF CRYSTALS
		Volatile substances	
dl-Amphetamine	Gold chloride in dild phosphoric acid Platinic chloride in dild phosphoric acid	direct or volatility volatility	Very irregular plates, with irregular blade-arms especially after evapn; square if perfect. Irregular blades and needles, very low birefringence; after evapn, characteristic plates with narrow irregular arms of blades.

32.227 *Characteristics of Microchemical Tests for Sympathomimetics*—Continued

SYMPATHOMIMETIC	REAGENT	TEST	DESCRIPTION OF CRYSTALS
d-Amphetamine	Gold chloride in dild phosphoric acid Platinic chloride in dild phosphoric acid	direct or volatility volatility	Long yellow rods and blades; with evapn, some crystals as with *dl* may form. Long needles, often bent, very little birefringence; after some evapn, long rectangular blades. (*l*-Ephedrine in direct test gives similar crystals which are more sol.; it is less volatile and does not normally form crystals in hanging drop.)
Epinephrine	Sodium tetraphenylboron	volatility	$MeNH_2$ liberated; birefringent X's or 4-arm crystals; also thick blades with central rib, pointed ends, positive elongation.
Isoproterenol	Sodium tetraphenylboron	volatility	Isopropylamine liberated; plates tending to nonregular hexagons; no birefringence where plates lie flat but there are rods which are birefringent.
d- and *dl*-Methamphetamine	Gold chloride in dild phosphoric acid Platinic chloride in dild phosphoric acid	direct or volatility volatility	Long blades and jointed crystals, fairly high birefringence. Grains with sharp edges which aggregate in chains and short prisms. Birefringent.
d-Methamphetamine	Bismuth iodide in dild sulfuric acid	volatility	Drops, long orange splinters, blades, needles; also deep red angular grains (red prisms only after evapn.)
dl-Methamphetamine	Bismuth iodide in dild sulfuric acid	volatility	Drops, crystallizing in orange-red prisms with conspicuously slanting ends; inclined extinction ca 20°; also "mossy" formation of grains and some large deep red grains.

Slightly volatile substances

dl-Ephedrine (racephedrine)	Gold chloride in dild phosphoric acid Bismuth iodide in dild sulfuric acid	direct or volatility volatility	Irregular plates based on the square growing along diagonals in 4 arms; some birefringent, some not. Orange rods or sticks, short and stubby, some plates; more irregular plates on evapn.
l-Ephedrine	Gold chloride in dild phosphoric acid Bismuth iodide in dild sulfuric acid	direct or volatility volatility	Long needles or splinters and long jointed forms; strong birefringence. Long brownish-orange needles, often branching or in sheaves; also, especially with evapn, orange irregular blades.
Pseudoephedrine	Gold chloride in dild phosphoric acid	direct or volatility (2 hr)	Thin branching sticks, many like combs; some broaden to blades or spear-head plates; very high birefringence.
Phenylpropanolamine	Gold chloride in dild phosphoric acid	direct volatility (2 hr)	Plates and blades of extremely high birefringence, elongate hexagonal or diamonds, very bright colors. Branch into 4 or 6 irregular arms. After definite drying, pyramidal grains to blades and plates with irregular arms, very birefringent.
Phenmetrazine	Gold chloride in dild phosphoric acid Bismuth iodide in dild sulfuric acid	direct or volatility volatility	Rectangular plates joined in jagged arms of strongly birefringent crystals, often in X forms, very characteristic. Orange-red blades, usually pointed ends, often in rosettes; also with needles in branching aggregates; also red prisms.

For Synthetics—Official

32.228 REAGENTS

(a) *Acetic acid.*—Dil. 6 ml HOAc to 100 ml with H_2O.

(b) *Ammoniacal nickel acetate soln.*—Mix 1 vol. 5% $Ni(OAc)_2.4H_2O$ soln with 1 vol. NH_4OH (2+3). Use clear supernatant.

(c) *Ammoniacal silver nitrate soln.—See* **32.221(a)**.

(d) *Ammonium thiocyanate soln.—See* **32.221(c)**.

(e) *Barium hydroxide soln.*—Satd aq. soln.

(f) *Benzaldehyde.*—N.F. X quality.

(g) *Bismuth iodide soln.—See* **32.221(d)**.

(h) *Bromide-bromate soln.*—Dissolve 0.3 g $KBrO_3$ and 1.2 g KBr in H_2O, and dil. to 100 ml.

(i) *Glycerol-alcohol mixture.*—(1+1).

(j) *Gold bromide in hydrochloric acid soln.—See* **32.221(g)**.

(k) *Gold chloride soln.—See* **32.221(h)**.

(l) *Iodine potassium iodide soln.—See* **32.221(j)**.

(m) *Lead acetate soln.*—Dissolve 5 g $Pb(OAc)_2.3H_2O$ in H_2O and dil. to 100 ml.

(n) *Lead triethanolamine soln.*—Add 1 ml triethanolamine (tech. 90% is satisfactory) to soln of 1 g $Pb(OAc)_2.3H_2O$ in 20 ml H_2O. Slight turbidity does not interfere.

(o) *Magnesia mixture.*—Dissolve 55 g $MgCl_2.6H_2O$ and 140 g NH_4Cl in H_2O. Add 130.5 ml NH_4OH and dil. to 1 L with H_2O.

(p) *Mercuric chloride soln.—See* **32.221(l)**.

(q) *Mercurous nitrate soln.*—Dissolve 15 g $HgNO_3.H_2O$ in mixt. of 90 ml H_2O and 10 ml HNO_3 (1+9). Preserve in dark, amber bottle contg small globule of Hg.

(r) *Nitric acid.*—(1+1).

(s) *Phosphotungstic acid soln.*—Dissolve 5 g $P_2O_5.24WO_3.xH_2O$ in 100 ml H_2O.

(t) *Picric acid.*—Crystals.

(u) *Picrolonic acid soln.*—Dissolve 250 mg 1-(*p*-nitrophenyl)-3-Me-4-nitropyrazolone in 25 ml alcohol.

(v) *Platinic chloride soln.—See* **32.221(n)**.

(w) *Potassium cadmium iodide soln.—See* **32.221(o)**.

(x) *Potassium ferrocyanide soln.—See* **32.221(p)**.

(y) *Silicotungstic acid soln.*—Dissolve 5 g $4H_2O.SiO_2.12WO_3.22H_2O$ in 100 ml ca 6*N* H_2SO_4.

(z) *Silver nitrate soln.*—Dissolve 1 g $AgNO_3$ in 20 ml H_2O.

(aa) *Sodium nitrite soln.*—Dissolve 10 g $NaNO_2$ in H_2O and dil. to 100 ml.

(bb) *Zinc pyridine soln.*—Add 1 ml pyridine to soln of 1 g $Zn(OAc)_2.2H_2O$ in 20 ml H_2O.

32.229 *Characteristics of Microchemical Tests for Synthetics*

SYNTHETIC	SOLVENT	CONCENTRATION OF SYNTHETIC	REAGENT	DESCRIPTION OF TESTS AND CRYSTALS
Acetanilid (*117*)	HCl (1+3)	1:100	Phosphotungstic acid	Rosettes of prisms.
	HCl (1+3)	1:100	Bromide-bromate soln	Small prisms.
Acetophenetidin (*117*)		Ca 1 mg powd. material	HNO_3	Add 1 drop HNO_3, let stand few sec., then add 1 drop H_2O. Bright yellow, curving, branched crystals.
	HCl (1+3)	Satd soln	Iodine potassium iodide	Large, irregular plates.
Acetylsalicylic acid (*118*)	2% triethanolamine	1:50	Silver nitrate	Fine, curling, hair-like crystals form first near edge of drop.
Aminopyrine (*119*)	H_2O	1:100	Mercuric chloride	Long, slender, radiating crystals, often curved.
			Potassium cadmium iodide	Groups of spiny branches.
Amytal ® (*120*) (Amobarbital)	NH_4OH (1+9)	1:50	Acetic acid	Long, branching needles; some hexagonal plates in groups.
	NH_4OH (1+9)	1:25	Acetic acid	Groups of rectangular plates.
Antipyrine (*121*)	H_2O	1:100	Potassium ferrocyanide	Add 1 drop HCl (1+39). Acicular and prismatic crystals form.
Barbital (*120*)	—	Ca 1 mg powder	Ammoniacal silver nitrate	Stir to aid soln and crystn. Very small, twinned crystals and larger tufts.
	NH_4OH (1+9)	1:50	Acetic acid	Dark burrs (stirring hastens crystn).

32.229 *Characteristics of Microchemical Tests for Synthetics*—Continued

Synthetic	Solvent	Concentration of synthetic	Reagent	Description of tests and crystals
Benzoic acid (*118*)	—	Dry powder	Lead triethanolamine	Stir small quantity of synthetic into 1 drop reagent. Stir thoroly to induce crystn. 4-sided plates, singly and in groups.
	—	Dry powder	Zinc pyridine	Stir small quantity of synthetic into 1 drop reagent. Stir thoroly to induce crystn. Hexagonal crystals.
	2% triethanolamine	1:100 to 1:200	Silver nitrate	Rods or curving blades with irregular ends.
Cinchophen (*100*)	0.1*N* NaOH Add H_2O, and make slightly acid with HCl	1:1000	Gold chloride	Dark clusters of needles. Few short, rhombic crystals.
Diallylbarbituric acid (*122*)	—	Dry powder	Lead triethanolamine	Stir small quantity of synthetic into 1 drop reagent. Rods singly and in clusters.
	—	Dry powder	Barium hydroxide	Stir small quantity of synthetic into 1 drop reagent. Rods singly and in groups.
Dinitrophenol (*119*)	Small quantity of 0.1*N* NaOH	1:100	HCl	Plates with 4 branches. In more dil. soln, single rectangular plates.
Diphenhydramine hydrochloride (Benadryl hydrochloride ®) (*123*)	Glycerine-alcohol (1+1) or H_2O	Ca 0.2 mg powder or tablet material or 1:1000	Platinic chloride	Aggregates of platy crystals form readily in glycerin-alcohol, gradually in H_2O. Plates with jagged edges, tendency to twin, forming X-shaped aggregates, hour-glass forms, and dendritic structures. First order gray polarization colors; symmetrical or parallel extinction. Plates show positive elongation.
Ethyl aminobenzoate (Benzocaine) (*100*)	0.1*N* HCl	1:100	Potassium ferrocyanide	Colorless, irregular plates and rods.
Hydroxyquinoline sulfate (Chinosol ® Oxyquinoline) (*100*)	Dissolve salt in H_2O. Dissolve free base in HCl (1+3), avoiding excess	1:500	Magnesia mixt.	Small, elliptical grains. Few burr-shaped crystals on standing.
Mandelic acid (*122*)	H_2O	1:100	Lead acetate	Rosettes of thin, curving plates.
	H_2O	1:100	Mercurous nitrate	Burr-shaped groups of needles.
Methenamine (*121*)	H_2O	1:500	Silicotungstic acid	Thin, transparent, rectangular crystals.
Metrazol ® (Pentylenetetrazol) (*99*)	H_2O	—	Mercuric chloride (1:10)	Rods, many almost needle-like; frequently in groups; also in radiating aggregates.
	H_2O	1:100	Silicotungstic acid	Amorphous, changes to elongated prisms; also long needles.
Neocinchophen (*117*)	HCl (1+3)	Satd soln	Ammonium thiocyanate	Rosettes of needles (Gentle agitation by tipping slide back and forth hastens crystn.)
	HCl (1+3)	Satd soln	Platinic chloride	Needles in clusters.

32.229 *Characteristics of Microchemical Tests for Synthetics*—Continued

SYNTHETIC	SOLVENT	CONCENTRATION OF SYNTHETIC	REAGENT	DESCRIPTION OF TESTS AND CRYSTALS
Phenobarbital (*120*)	—	Ca 1 mg powder	Ammoniacal nickel acetate	Stir to aid soln and crystn. Single rectangular crystals.
Pyranisamine maleate (Pyrilamine) (*123*)	Glycerine-alcohol (1+1) or H_2O	1:1000 or ca 0.1 mg powder	Platinic chloride	Needles in rosette aggregates, sheaves, and singly. Needles show second order blue and green, and first order red and yellow polarization colors; parallel extinction and negative elongation.
Pyridium ® (Mallophene ®) (*100*)	Dissolve salt in H_2O. Dissolve free base in HCl (1+3), avoiding excess	1:1000	Ammonium thiocyanate	Small, red-brown, dense sheaves.
Salicylic acid (*118*)	HCl (1+3)	Dry powder	Bromide-bromate soln	Stir few crystals into 1 drop of the HCl. Add 1 drop reagent. Fine needles appear to grow from the crystals of salicylic acid.
	—	Dry powder	Lead triethanolamine	Stir few crystals into 1 drop reagent. Rods or needles grow from the crystals of salicylic acid.
	2% triethanolamine	1:100 to 1:200	Silver nitrate	Small, irregular plates; few short rods.
Sulfadiazine (*104*)	H_2O	—	Gold bromide in HCl	Red, circular masses composed of fine needles.
Sulfanilamide (*122*)	—	Dry powder	Benzaldehyde	Stir thoroly small quantity into 1 drop reagent. 4-sided plates.
	0.1*N* HCl	Satd soln	Sodium nitrite	Yellow needles.
Sulfapyridine (*107*)	Acetone $+H_2O$	—	Gold chloride	Yellow rods or blades; also X-shaped aggregates.
Sodium sulfapyridine monohydrate (*107*)	H_2O	1:100	Gold chloride	Yellow rods in X-shaped aggregates.
Sulfathiazole (*99*)	50% alcohol	—	Picric acid	Long, fine, yellow needles, many curved, occur in dense rosettes; also short, stout rods in groups or singly.
	50% alcohol (or no solvent)	—	Picrolonic acid	Distinct rosettes of very fine needles; also single needles.
Triethanolamine (*121*)	H_2O	1:100	Bismuth iodide	Oily globules changing to large, red, hexagonal plates and prismatic crystals.
Tripelennamine hydrochloride (Pyribenzamine hydrochloride ®) (*123*)	Glycerine-alcohol (1+1) or H_2O	1:1000 or ca 0.1 mg powder or tablet material	Platinic chloride	Small needles and bladed crystals in dense rosette aggregates and singly. Needles show first order white and yellow polarization colors, parallel extinction, and positive elongation.

For Xanthine Group Alkaloids (116)—First Action

32.230 REAGENTS

(a) *Bismuth iodide soln.—See* **32.221(d)**(2).

(b) *Gold bromide in dilute hydrochloric acid.*—Dissolve 1 g $HAuCl_4.3H_2O$ in 1.5 ml 40% HBr and add HCl (1+3) to make 45 ml.

(c) *Iodine potassium iodide soln (5–14).*—Dissolve 5 g I and 14 g KI in H_2O and dil. to 100 ml with H_2O.

32.231 GENERAL TEST

(Murexide reaction)

To small amount of substance in small porcelain crucible add very small crystal $KClO_3$ and 1 drop HCl (1+1). Set on hot plate at ca 100°, or hot enough to boil off H_2O in short time. Soon after drying, residue becomes orange to red. Add 1 drop NH_4OH. Purple color is produced in presence of caffeine, theobromine, theophylline, and related xanthine derivatives.

32.232 IDENTIFICATION

(a) *Bismuth iodide soln.*—Add 1 drop reagent to little dry material on slide and cover.

(b) *Gold bromide in dilute hydrochloric acid.*—Place 1 drop reagent beside very small amount of dry substance on slide and apply cover glass so that reagent flows over substance.

(c) *Iodine potassium iodide soln. (5–14).*—In depression of cavity slide dissolve little of substance in small drop 1% NaOH soln and stir in excess $NaHCO_3$ (some undissolved). Add large drop reagent and stir slightly. Add several crystals KCl. Examine center and edge as soln evaps.

32.233 *Characteristics of Microchemical Tests for Xanthine Alkaloids*

ALKALOID	REAGENT	DESCRIPTION OF CRYSTALS
Caffeine	Gold bromide in dil. HCl	Outer part: brownish needles with bright white birefringence. Inside part: small rods to sticks, little grains and plates with weak yellow birefringence.
	Bismuth iodide	Small brownish-orange rods or blades growing from sample or nearby in rosettes; also some orange grains.
	Iodine potassium iodide (5–14)	Grains, dark red to black, sometimes yellow or orange-brown; generally square or cubical; birefringent with fairly strong light; some irregular dichroic blades.
Dyphylline	Gold bromide in dil. HCl	Needles, scattered and in rosettes; fairly bright birefringence.
	Bismuth iodide	Very small grains, flakes, blades in multitudes, birefringent.
	Iodine potassium iodide (5–14)	Fuzzy brown dense rosettes thruout drop, birefringent around rims; excess reagent must be used; 5 min. required to form crystals.
Theobromine	Gold bromide in dil. HCl	Grains or plates in dense groups; bright birefringence at edge of cluster.
	Bismuth iodide	Brown needles in rosettes.
	Iodine potassium iodide (5–14)	Orange brown chips; also rectangular plates with opposite sides incised; smaller crystals: grains, often lens shaped or diamonds; birefringent, somewhat dichroic.
Theophylline	Gold bromide in dil. HCl	Long needles in sheaves; fairly bright birefringence.
	Bismuth iodide	Grains and short prisms, often rectangular; brightly birefringent.
	Iodine potassium iodide (5–14)	Black needles in rosettes around edge; birefringent; when larger, blades or rods, dichroic black vertically to yellow horizontally.

Optical-Crystallographic Examination of Crystalline Substances—First Action

(General knowledge of microscopy and crystallography is necessary for application of this technic. Some of standard works on this subject are listed in Selected References (*124*). Optical-crystallographic properties of antihistamines, barbiturates, sulfonamides, and sympathomimetic amines are given in Tables **43.029** and **43.030**.)

32.234 APPARATUS

(a) *Polarizing microscope.*—Fitted with polarizing prisms below and above rotating, graduated circular stage and with accessories (Bertrand lens or pinhole eyepiece, first order red or quartz wedge compensators) for observation of interference figures, optic sign, and sign of elongation.

(b) *Refractometer.*—For measuring refractive indices of liquids at 20° from 1.300–1.840 with accuracy of ±0.0005.

32.235 REAGENTS

Immersion media.—Ideally immersion media for refractive index detn should have same color and intensity of color as substance being examined and be chemically stable. Refractive indices should not vary perceptibly with ordinary changes of temp. with exception of special liquids used in index-variation methods. Permanent set of liquids covering range 1.430–1.790 in 0.005 intervals made with following mixts is useful for both inorg. and org. substances:

Mixture	n_D
Kerosene and mineral oil	1.435–1.480
Mineral oil and α-monochloronaphthalene	1.485–1.640
α-Monochloronaphthalene and methylene iodide	1.645–1.740
Methylene iodide and sulfur	1.740–1.790

Substances sol. in these liquids require prepg special set of liquids.

32.236 DETERMINATIONS

Refractive indices.—Det. refractive indices by mounting cryst. material in suitable immersion liquids and observing Becke line. Successively suspend crystals or crystal fragments of substance in immersion liquids of known refractive indices. Greater the difference between refractive indices of crystal and liquid, the more prominently one stands out in bold relief from other. By repeatedly mounting such crystals in oils of successively lower or higher index, ultimately zone of contact of crystal and liquid becomes practically invisible, demonstrating that refractive indices of liquid and solid have been matched.

In case of substances crystg in isometric (cubic) system, there is only 1 refractive index, designated by n. Such substances are not doubly refractive when examined with crossed nicols. Substances crystg in other systems, hexagonal, tetragonal, monoclinic, triclinic, and orthorhombic, in ideal cases, have more than 1 measurable refractive index. With uniaxial substances such as those crystg in hexagonal and tetragonal systems, 2 significant indices can be detd, designated as n_e and n_ω. Substances crystg in monoclinic, triclinic, and orthorhombic systems, in ideal cases, have 3 refractive indices designated as n_α, n_β, and n_γ.

Extinction and extinction angle of anisotropic substances.—Anisotropic crystals, when rotated through 360° on stage, become dark 4 times. Positions of darkness are known as extinction positions and correspond to positions in which vibrations of birefringent rays produced by crystal are mutually parallel to vibration directions of polarizer and analyzer indicated by cross hairs in eyepiece. If crystal extinguishes when crystal edge or face is parallel to one of cross hairs, extinction is *parallel.* If bisector of silhouette angle is parallel to one of cross hairs, extinction is *symmetrical.* Crystals showing extinction differing from these 2 have *inclined* extinction. Measure extinction angles on those crystals showing inclined extinction by rotating crystal so that crystal edge or face is parallel to 1 of cross hairs. Rotate stage until crystal extinguishes. Read on stage vernier extinction angle between face or edge at extinction and nearest cross hair. Express extinction angles with relationship to principal vibration directions of light and crystallographic axes.

Elongation.—Many crystals are frequently elongated in 1 direction. Relationship between direction of elongation and vibration directions of slow and fast rays of anisotropic crystal is sometimes of determinative value. If substance is length slow, *i.e.*, slow ray or higher refractive index is parallel to direction of elongation, sign of elongation is positive; if substance is length fast, sign is negative.

Sign of elongation (+ or −) is detd with gypsum plate and crossed nicols. A long and narrow crystal, showing very little color with crossed nicols, is so oriented that its long dimension is parallel to direction "z" of plate (slow ray) which is inserted in slit of microscope tube. (Direction "z" is indicated by arrow on plate.) If crystal appears blue or other color of higher order than red-violet due to plate, elongation is +; if crystal appears yellow, white, or gray, *i.e.*, of lower order color than red-violet field, elongation is −.

Optic character and optic sign.—Det. optic character (uniaxial or biaxial) and optic sign (+ or −), using first order red or quartz wedge compensators in conjunction with interference figures. Obtain interference figures from conoscopic images of crystals suitably oriented. In absence of interference figures, det. these properties from relationship of principal refractive indices. When $(n_\beta - n_\alpha)$ is less than $(n_\gamma - n_\beta)$, optic sign is +. When $(n_\beta - n_\alpha)$ is greater than $(n_\gamma - n_\beta)$, optic sign is −.

Optic axial angle (2V).—Calc. axial angle (2V) from values of 3 refractive indices (here designated α, β, and γ) according to formulas:

$$\mathrm{Cos}^2\, V_\alpha = \frac{\gamma^2(\beta^2 - \alpha^2)}{\beta^2(\gamma^2 - \alpha^2)} \text{ (for − optic sign), or}$$

$$\mathrm{Cos}^2\, V_\gamma = \frac{\alpha^2(\gamma^2 - \beta^2)}{\beta^2(\gamma^2 - \alpha^2)} \text{ (for + optic sign),}$$

where $2V_\alpha$ is axial angle about α, and $2V_\gamma$ is axial angle about γ. Alternatively, estimate approx. value of 2V from curvature of isogyre referring to diagrams of substances with known angles.

MISCELLANEOUS DRUGS

32.237 Benzoic and Salicylic Acids in Ointments (*125*)—Official

Weigh accurately ca 2.5 g sample into separator, add ca 50 ml ether, and swirl until sample dissolves. Completely ext. with satd $NaHCO_3$ soln, using 15, 15, 10, and 10 ml portions, or more. Ext. combined $NaHCO_3$ solns with 10 ml $CHCl_3$ and discard $CHCl_3$. Acidify with HCl and ext. with $CHCl_3$-ether (2+1) until benzoic and salicylic acids are completely extd. Filter exts into 250 ml beaker thru filter moistened with $CHCl_3$. Evap. to ca 5 ml on steam bath, using air current; then complete evapn at room temp.

Dissolve residue in ca 20 ml dild alcohol (ca 50%); carefully titr. with 0.1*N* NaOH, using phthln; record vol., and add ca 2 ml excess. Completely evap. alcohol on steam bath, using air current. (Evapn from ca 50 ml vol. to 5 or 10 ml is sufficient. Alcohol consumes Br.)

Transfer remaining titrn liquid and washings to 100 ml vol. flask, cool to room temp., and dil. to mark with H_2O. Mix thoroly. Pipet 25 ml aliquot into I flask; add 25 ml H_2O, exactly 25 ml 0.1*N* KBr-$KBrO_3$, **32.128**, and ca 5 ml HCl. Swirl mixt. frequently during 30 min. Carefully add 5 ml KI soln (ca 10%), shake well, and in ca 1 min. titr. with 0.1*N* $Na_2S_2O_3$, using starch indicator.

Calc. salicylic acid from the 0.1*N* KBr-$KBrO_3$ consumed; 1 ml 0.1*N* KBr-$KBrO_3$ = 0.0023 g salicylic acid. Calc. benzoic acid from difference between 0.1*N* NaOH titrn value and 0.1*N* NaOH equiv. of salicylic acid found. 1 ml 0.1*N* NaOH = 0.01221 g benzoic acid or 0.01381 g salicylic acid.

32.238 Cod Liver Oil in Emulsions (*126*)—Official

Weigh, into tared 150 ml beaker, enough well-mixed sample to contain ca 2 g cod liver oil. Add ca 10 g finely powd. $CaCO_3$ and mix thoroly with stirring rod. Add 30 ml $CHCl_3$, mix thoroly, and decant thru dry filter into 100 ml air-dried, tared beaker. Continue to ext. and wash repeatedly with 5–10 ml portions $CHCl_3$ until filtrate is ca 60 ml. Evap. $CHCl_3$ on steam bath with air current to ca 5 ml.

Continue extn and carefully wash paper and funnel, filtering into 250 ml beaker until filtrate is ca 150 ml. Evap. to ca 10 ml and transfer to first tared beaker. Repeat procedure until extn is complete or until 25 ml solvent upon evapn in second tared beaker yields 1 mg or less of residue. Evap. $CHCl_3$ in first tared beaker and keep on steam bath ca 10 min. after $CHCl_3$ odor disappears. Dry at not >100° for 5 min. intervals to constant wt or until loss is 1 mg or less.

Caution: Avoid prolonged heating or long exposure to air at room temp. Oil absorbs O, wt increases appreciably, and physical constants change.

Iodoform (*127*)—Official

32.239 REAGENTS

(**a**) *Ammonium thiocyanate soln.*—0.05*N*. Stdze against 0.1*N* $AgNO_3$, using equal vol. alcohol and 3 ml $FeNH_4(SO_4)_2$ soln as indicator.

(**b**) *Ferric ammonium sulfate indicator.*—Dissolve 8 g $FeNH_4(SO_4)_2.12H_2O$ in 100 ml H_2O.

32.240 DETERMINATION

Weigh accurately ca 0.25 g CHI_3 and transfer quantitatively to 200 ml erlenmeyer. Add 40 ml alcohol, swirl gently until CHI_3 dissolves, filter if necessary, and immediately add 40 ml 0.1*N* $AgNO_3$ and 10 ml HNO_3. Swirl gently ca 5 min., let stand at room temp. 2–3 hr, and then swirl occasionally as aid in flocculating the AgI. Titr. excess $AgNO_3$ with 0.05*N* NH_4CNS, using 3 ml of the $FeNH_4(SO_4)_2$ indicator. 1 ml 0.1*N* $AgNO_3$ = 0.01313 g CHI_3. Or: filter, collecting AgI on dried and weighed gooch, wash with H_2O and finally with alcohol, and dry to constant wt at ca 125°. 1 g AgI = 0.5590 g CHI_3.

32.241 Iodoform in Ointments (*128*)—Official

Transfer ca 2.5 g sample to tared 50 ml beaker and weigh. Add 5 ml $CHCl_3$, stir gently with glass rod, and transfer bulk of undissolved ointment and $CHCl_3$ soln to 250 ml g-s. flask. Add 5 ml $CHCl_3$ to ointment remaining in beaker and stir until dissolved. Add soln to flask and finally wash beaker 3 times, using not >5 ml $CHCl_3$ each time, and add washings to flask. Or: weigh sample in small, tared glass capsule, drop capsule with contents into 250 ml g-s. flask, and add not >20 ml $CHCl_3$. (Use glass capsule only in volumetric detn.) Swirl gently until all ointment dissolves. Add 40 ml 0.1*N* *alc.* $AgNO_3$ and swirl to wash down any CHI_3 that adheres to sides of flask. Slowly add 10 ml HNO_3 and let stand at room temp. ca 18 hr. Titr. excess of 0.1*N* alc. $AgNO_3$ with 0.05*N* NH_4CNS, **32.239(a)**, using 3 ml $FeNH_4(SO_4)_2$ indicator, **32.239(b)**, shaking mixt. vigorously near end of titrn. 1 ml 0.1*N* $AgNO_3$ = 0.01313 g CHI_3.

For gravimetric detn use ordinary erlenmeyer instead of g-s. flask. Weigh ointment base into 100 ml beaker and add $CHCl_3$. After ointment base dissolves, filter thru gooch, using suction. Wash beaker and crucible once with alcohol. Wash crucible several times with $CHCl_3$ without suction. Collect filtrate in erlenmeyer and add 40 ml 0.1*N* $AgNO_3$ and 10 ml HNO_3 in small portions. Let mixt. stand 18 hr. Collect AgI on weighed gooch, using suction. Wash with H_2O and then with alcohol. Finally wash repeatedly

with $CHCl_3$ without suction. Dry gooch and contents at ca 125° to constant wt. 1 g AgI = 0.5590 g CHI_3.

32.242 Iodoform on Gauze (*129*)—Official

Weigh, in tared g-s. weighing bottle, sample of CHI_3 gauze contg ca 1 g CHI_3. (CHI_3 gauze is usually moist and loses wt rapidly when exposed to air.) Transfer to 150 ml beaker, add ca 75 ml alcohol, and stir until CHI_3 dissolves. Filter into 200 ml vol. flask, draining alc. soln by pressing on gauze. Wash with four or five 25 ml portions alcohol, filter washings, and finally dil. to vol. with alcohol. Pipet 40 ml aliquot into 200 ml erlenmeyer and immediately add 40 ml 0.1*N* $AgNO_3$ and 10 ml HNO_3. Proceed as in **32.241**, beginning "let stand at room temp. ca 18 hr."

Mandelic Acid (*130*)—Official

32.243 QUALITATIVE TESTS

(Applicable to free acid)

See Microchemical Tests, **32.229**.

32.244 DETERMINATION

(**a**) *Tablets.*—Weigh quantity of powd. sample contg 0.4–0.5 g mandelic acid and transfer to separator contg 10 ml H_2O. Acidify with HCl (1+3) and add 2 ml excess. Ext. with six 20 ml portions $CHCl_3$-ether (2+1); wash each portion in second separator with 2 ml H_2O, and pass soln thru cotton plug, previously satd with solvent, into 250 ml beaker. Wash outer surface of separator stem with few ml solvent and add to main portion. Test for complete extn with 15 ml more of solvent and evap. in sep. beaker. Wash any residue thus obtained into beaker contg main ext. with few ml solvent. Evap. to dryness at not >40° with aid of fan. Dissolve residue in 25 ml CO_2-free H_2O and titr. with 0.1*N* NaOH, using phthln. 1 ml 0.1*N* NaOH = 0.01521 g mandelic acid, $C_6H_5CHOHCOOH$; 0.01691 g NH_4 mandelate, $C_6H_5CHOHCOONH_4$; 0.01741 g Na mandelate, $C_6H_5CHOHCOONa$; 0.01711 g Ca mandelate, $(C_6H_5CHOHCOO)_2Ca$; and 0.01632 g Mg mandelate, $(C_6H_5CHOHCOO)_2Mg$.

After titrn, mandelic acid may be re-extd and ext. used for m. p. detns or qual. tests.

(**b**) *Liquid preparations.*—Measure 1 ml sample, or aliquot of diln contg 0.4–0.5 g mandelic acid, into separator and acidify with HCl (1+3). Proceed as in (**a**).

Mannitol Hexanitrate; Mannitol Hexanitrate and Phenobarbital (*131*)—Official

(*See* also **32.255–32.258**)

32.245 REAGENTS

(**a**) *Phenoldisulfonic acid.*—Heat 5 g colorless phenol, 30 ml H_2SO_4, and 15 ml fuming H_2SO_4 (ca 20% free SO_3) on steam bath 2 hr.

(**b**) *Nitrate std soln.*—Dissolve 100 mg KNO_3 or $NaNO_3$ in ca 1 ml H_2O and dil. to 100 ml with HOAc.

(**c**) *Phenobarbital std soln.*—In 100 ml vol. flask dissolve 100 mg phenobarbital and dil. to vol. with HOAc. Pipet 5 ml of this soln and 15 ml HOAc into 100 ml vol. flask, dil. to vol. with H_2O, and filter, discarding first 5 ml filtrate.

32.246 PREPARATION OF SAMPLE

Transfer accurately weighed sample contg ca 30 mg mannitol hexanitrate to 50 ml vol. flask, and dil. to vol. with HOAc. Shake well and filter, discarding first 5 ml filtrate.

32.247 DETERMINATION OF MANNITOL HEXANITRATE

Treat 1.0 ml sample, 1.0 ml std, and 1.0 ml HOAc blank in identical manner. Transfer 1 ml soln and 2 ml phenoldisulfonic acid to 100 ml vol. flask and let stand 15 min. Dil. with H_2O to ca 60 ml, add NH_4OH (ca 10 ml) until max. yellow color appears, cool to room temp., dil. to mark with H_2O, and mix. Det. absorbances of sample and std relative to blank at 408 mμ.

$(A_1 \times R_2 \times k \times 50)/(A_2 \times R_1) = \%$ mannitol hexanitrate, where A_1 is absorbance of sample, A_2 is absorbance of std, R_1 is mg sample, R_2 is mg std/ml, and k is 88.65 for $NaNO_3$ and 74.56 for KNO_3 std.

32.248 DETERMINATION OF PHENOBARBITAL

Pipet 10 ml aliquot sample soln into 50 ml vol. flask, dil. to vol. with H_2O, shake, and filter, discarding first 5 ml filtrate. Prep. blank by dilg 10.0 ml HOAc to 50 ml with H_2O and filtering. Dil. sep. 20 ml aliquots of std, sample, and blank solns to 100 ml with NH_4OH (1+9), adjusting to room temp. before dilg to vol. (Final pH of soln, 9.0–9.6.) Det. absorbance of sample and std relative to blank at 240 mμ, and calc. phenobarbital content.

Methenamine (Hexamethylenetetramine) in Tablets (*132*)—Official

32.249 REAGENT

Modified Nessler reagent.—(1) Dissolve 10 g $HgCl_2$, 30 g KI, and 5 g acacia in 200 ml H_2O, and filter thru cotton; (2) dissolve 15 g NaOH in 100 ml H_2O. Mix 20 ml soln (1) with 10 ml soln (2).

32.250 DETERMINATION

Weigh 0.5 g powder, prepd as in **32.002**, into round-bottom flask, and add 100 ml H_2O and 25 ml HCl (1+2.5). Connect with reflux condenser (preferably of worm type) and boil gently 15 min. Cool, wash condenser tube with little H_2O, trans-

fer contents of flask to 250 ml vol. flask, and dil. to mark.

Chill 30 ml of the Nessler reagent and add 10 ml aliquot of the hydrolyzed soln of sample. Wash neck of container with jet of H_2O and let stand at least 1 min. Add 10 ml HOAc (1+1.5) so that inside of neck is completely washed by reagent, mix quickly and thoroly by rotating and tilting flask, and immediately add 20 ml 0.1*N* I from buret or pipet. Titr. excess I with 0.1*N* $Na_2S_2O_3$, adding 5–10 drops starch indicator, **4.004(f)**, toward end of titrn, until blue color disappears. Final color of soln is pale straw-green. If preferred, end point may be detd by reappearance of faint blue color by addn of drop of the I soln. 1 ml 0.1*N* I = 0.00117 g methenamine.

Methyl Salicylate (*133*)—First Action

32.251 REAGENTS

(**a**) *Salicylic acid std soln.*—20 mmg/ml. Dissolve 0.2500 g reagent grade salicylic acid in 95 ml $CHCl_3$ in 250 ml vol. flask and dil. to vol. with alcohol. Dil. 2.00 ml to 100 ml with alcohol.

(**b**) *Sodium bicarbonate soln.*—Dissolve 5 g $NaHCO_3$ in 100 ml of H_2O to which 1 drop HCl has been added.

32.252 DETERMINATION

Pipet 5.00 ml sample into 50 ml of ether-petr. ether mixt. (1+1) in separator and wash with two 5 ml portions cold, freshly prepd $NaHCO_3$ soln. Discard unemulsified aq. phases. Ext. org. layer with two 5 ml portions 5% NaOH soln followed by two 5 ml portions H_2O. Let phases sep. 5 min. and drain unemulsified aq. layers into another separator. Wash combined exts with 10 ml petr. ether and drain aq. phase into another separator. Acidify cautiously with HCl (litmus paper) and ext. with four 20 ml and one 15 ml portions of $CHCl_3$. Filter each ext. thru $CHCl_3$-moistened plug of cotton into 250 ml vol. flask. Dil. to vol. with alcohol and transfer 2.00 ml aliquot to 100 ml vol. flask. Dil. to vol. with alcohol and record absorbance from 250 to 400 mμ, using alcohol as reference. Compare absorbance at 308 mμ with that of std salicylic acid soln and calc. to Me salicylate. Ratio Me salicylate to salicylic acid is 1.1016.

Methylene Blue (Methylthionine Chloride) (*134*)—Official

32.253 PREPARATION OF SOLUTION

(**a**) *Foreign material absent.*—Weigh into 50 ml beaker 0.1–0.14 g powd. sample, **32.002,** and transfer to 200 ml vol. flask with 100–140 ml H_2O. Dissolve completely by heating 30 min. on steam bath with frequent shaking.

(**b**) *Oils or water-insoluble material present.*—Transfer to 150 ml beaker weighed quantity of prepd sample, **32.002,** contg 0.1–0.14 g methylene blue. Add 15 ml CCl_4, warm on steam bath few min., and stir with glass rod to dissolve oils. Transfer to 100 ml separator, using ca 50 ml hot H_2O and little CCl_4 if necessary. Cool, shake, and let sep. Transfer CCl_4 with undissolved material to second separator for further treatment. (Clear aq. soln of dye should now remain in first separator. If not clear, ext. with another 15 ml portion CCl_4, transferring any remaining insol. material in similar manner to second separator.) Add ca 10 ml CCl_4 to second separator and remove methylene blue by shaking vigorously with 20–40 ml portions H_2O until practically no more dye is extd. (Few drops of HOAc hastens this extn.) To aq. exts in 400 ml beaker add main soln from first separator, cover with inverted watch glass on glass rods, and evap. to ca 50 ml. Proceed as in (**c**). CCl_4 soln may be reserved for qual. tests for oils.

(**c**) *Water-soluble material present.*—Either use aq. soln from (**b**), or weigh portion of sample contg 0.1–0.14 g methylene blue into 150 ml beaker, add ca 50 ml H_2O, and heat 30 min. on steam bath with occasional shaking. Transfer to 100 ml separator, keeping vol. as small as possible. Ext. with *α-dichlorohydrin,* using 10, 5, 3, and 2 ml portions. Combine dichlorohydrin exts in 200–300 ml separator, add 3 or 4 times their vol. CCl_4, and ext. dye with H_2O by repeated vigorous shaking with 30–50 ml portions. (Few drops of HOAc hasten removal.) From combined aq. exts remove any traces of dichlorohydrin by shaking once with ca 15 ml CCl_4 and draining after settling 5–10 min. Evap. aq. exts to ca 50 ml over flame, covering beaker as in (**b**) with inverted watch glass. Transfer to 200 ml vol. flask. Dissolve completely by heating 30 min. on steam bath with frequent shaking.

32.254 DETERMINATION

Conduct blank as in detn, including filtration. Cool soln, **32.253**(**a**) or (**c**), add 50 ml HOAc, shake thoroly, and let stand at least 25 min. Add 30 ml 0.2*N* I from buret, adding first 10 ml by fast drops with constant rotating of flask and remaining 20 ml at full speed, and continue shaking. Stopper flask and let stand 50 min., shaking thoroly 5 or 6 times during interval. Dil. to mark with H_2O, shake, and let stand 10 min. longer. Filter rapidly thru dry, folded, 12 cm paper. Titr. 100 ml aliquot with 0.1*N* $Na_2S_2O_3$, with or without starch indicator as desired. Correct for blank titrn. 1 ml 0.2*N* I = 0.01495 g methylene blue, $C_{16}H_{18}N_3ClS.3H_2O$; or 0.01324 g anhyd. methylene blue, $C_{16}H_{18}N_3ClS$.

Nitrate Esters—First Action

Infrared Method (135)

(Applicable to mannitol hexanitrate, erythritol tetranitrate, or pentaerythritol tetranitrate)

32.255 APPARATUS

(a) *Recording infrared spectrophotometer.*—With two 1.0 mm liquid absorption cells with NaCl windows, preferably matched or of known absorbance difference, and KBr disk holder.

(b) *Chromatographic tube.*—25×200 mm with 5×40 mm stem.

(c) *Die and hydraulic press.*—Suitable for prepg KBr disks.

32.256 PREPARATION OF STANDARD SOLUTION

Ext. ester from commercial absorbate (usually 10% on lactose or other inert diluent) with ether, filter, and evap. to dryness with aid of air current at temp. not >50°. Dry in vac. desiccator 1 hr. Prep. std soln contg 0.5 mg ester/ml $CHCl_3$.

CAUTION: Pure crystalline nitrate esters are very explosive, especially pentaerythritol tetranitrate. Do not use sample contg >5 mg pure compound.

32.257 PREPARATION OF SAMPLE

Weigh at least 20 tablets and reduce to fine powder. Weigh sample contg ca 25 mg nitrate ester and transfer to 125 ml separator with ca 5 ml H_2O. Make distinctly acid with H_2SO_4 (1+9). Proceed as in (a) in absence of phenobarbital or (b) in presence of phenobarbital.

(a) Add 10 ml $CHCl_3$ to separator, shake vigorously several min., and let sep. Transfer $CHCl_3$ layer to 50 ml vol. flask. Ext. aq. soln with three addnl 10 ml portions $CHCl_3$ and transfer each ext. to the vol. flask. Dil. to vol. with $CHCl_3$, mix, and filter.

(b) Add 15 ml $CHCl_3$ to separator, shake vigorously several min., and let sep. Transfer $CHCl_3$ layer to chromatographic column contg 4 ml 1*M* K_3PO_4 soln adsorbed on 5 g Celite, collecting eluate in 50 ml vol. flask. Ext. aq. soln with three addnl 10 ml portions $CHCl_3$, and pass each ext. thru column, collecting eluate in the vol. flask. Dil. to vol. with $CHCl_3$, mix, and filter.

32.258 DETERMINATION

Transfer 5 ml aliquot $CHCl_3$ soln to 25 ml g-s. erlenmeyer and evap. to dryness with aid of air current at temp. not >50°. Add 5.00 ml $CHCl_3$ to residue, stopper flask tightly, and let stand 30 min. with occasional shaking to insure complete soln. Det. absorbance of std and sample solns against $CHCl_3$ at max. ca 6.0 μ and calc. amount of ester per tablet.

Evap. another portion $CHCl_3$ soln to dryness as above. Prep. KBr disk by grinding together in agate mortar 1 mg residue with 200 mg infrared spectrophotometric grade KBr and pressing in die and hydraulic press. Record spectrum from 2–15 μ and compare with spectrum of std nitrate ester to det. identity of sample.

Nitroglycerin (Glyceryl Trinitrate)

Reduction Method (136)—Official

32.259 APPARATUS

(a) *Connecting bulb.*—Hopkins style, ca 7.6 cm (3″) diam. This style has long inlet tube with opening on side of tube.

(b) *Condenser.*—Water-cooled, length 56 cm (22″), and preferably of Pyrex glass.

(c) *Adapter tube.*—Approx. 2.25 cm ($\frac{7}{8}$″) diam. at top and with narrow outlet.

(d) *Scrubber-trap.*—Any efficient trap in which all vapor is washed thoroly with H_2O before it leaves distg flask (*see* Fig. 66).

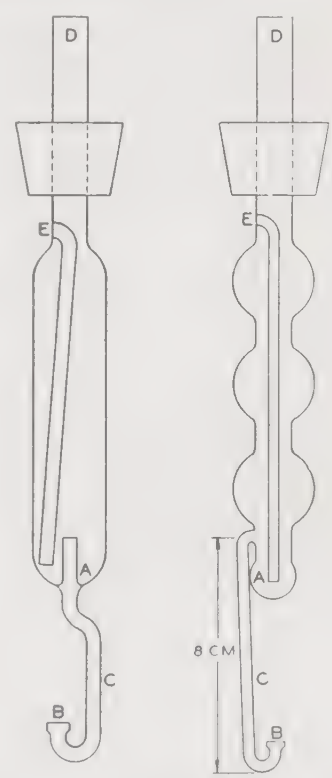

FIG. 66.—SCRUBBER TRAP FOR AMMONIA DISTILLATION

32.260 EXTRACTION

(a) *Ether extraction.*—Place in 50 ml beaker enough weighed sample to yield ca 0.0324 g nitroglycerin. If sample consists of tablets, count those taken; if of powd. material, mix thoroly before weighing. Add 10 ml ether, and to facilitate extn, reduce tablets to fine powder, using flat-end stirring rod. Stir thoroly. Decant ether thru dry 7 cm

quant. paper into 250 ml beaker contg 10 ml alcohol. Hold paper in place in funnel with the stirring rod and pour ether down rod. Make 4 addnl extns in same way. Dissolve ether-insol. residue in small quantity of H_2O, transfer soln to separator, and ext. with two 10 ml portions ether. Filter these exts, add to first exts, and evap. combined soln to ca 10 ml with fan. Transfer soln to 800 ml Kjeldahl flask, rinsing beaker first with 10 ml alcohol and then with little H_2O. Dil. to ca 300 ml with recently boiled and cooled NH_3-free H_2O.

(b) *Alcohol extraction.*—Weigh into g-s. erlenmeyer enough sample to yield ca 0.065 g (1 grain) nitroglycerin. If sample consists of tablets, count those taken; if of powd. material, mix thoroly before weighing portion taken for analysis. Pipet in 50 ml alcohol. To facilitate extn, reduce tablets to fine powder with flat-end rod. Stopper flask and shake. Let mixt. settle, transfer 25 ml aliquot of clear soln to 800 ml Kjeldahl flask, and dil. to ca 300 ml with NH_3-free H_2O.

32.261 DETERMINATION

Place flask on wire gauze with asbestos center. Add thru funnel 2 g *Devarda alloy*, ca 4 cm *heavy* (ca 16 gauge) *Al wire*, and 10–15 ml alc. KOH soln (15 g KOH dild to 100 ml with alcohol). Immediately after adding alkali, place little H_2O in scrubber trap (A and B, Fig. 66), and insert into neck of flask rubber stopper carrying scrubber trap and connecting bulb. Connect outlet tube of connecting bulb with condenser, which has been fixed in upright position and fitted with adapter tube dipping to bottom of 500 ml erlenmeyer contg measured vol. (ca 25 ml) 0.02*N* HCl or H_2SO_4 and 10–15 ml H_2O, and so inclined that tip of adapter is submerged as far as practicable under surface of liquid in flask.

Heat distn flask ca 1 hr, using small flame and regulating heat so that rapid evolution of H, but no appreciable distn, takes place. Gradually increase heat until distn begins; when active foaming ceases, continue distn with large flame until ca 40 ml liquid remains in distg flask. Lower flame toward end of distn to avoid cracking flask. Remove receiver contg distillate, add enough Me red, **32.023**(b), to make soln red, and titr. excess acid with 0.02*N* NaOH soln. From difference between this excess and quantity added, after making correction shown by blank test with same quantity of reagents distd in same manner, calc. % nitroglycerin in sample. 1 ml 0.02*N* acid = 0.001514 g nitroglycerin.

Infrared Method (137)—First Action

32.262 REAGENT

Nitroglycerin std.—Absorbate on lactose contg ca 10% nitroglycerin. Stdze by **32.259–32.261**. This product is stable indefinitely in tightly stoppered bottle.

32.263 DETERMINATION

Transfer to small separator number of tablets equiv. to ca 5 mg nitroglycerin. Dissolve or suspend in 5 ml H_2O, add 20 ml CS_2, shake 1 min., and let sep. Filter CS_2 layer thru pledget of cotton previously washed with CS_2 and collect in 100 ml beaker. Repeat extn with three 10 ml portions CS_2. Evap. combined exts to ca 3 ml, using gentle current of air, at temp. not $>50°$. Transfer quantitatively to 5 ml vol. flask and dil. to vol. with CS_2.

Accurately weigh quantity of std absorbate contg ca 5 mg nitroglycerin. Transfer to small separator and ext. as above.

Det. baseline absorbance, A_B, of sample and std solns relative to CS_2 at 7.89 μ, drawing baseline between min. at 7.5 and 8.3 μ. Calc. nitroglycerin content of sample. Record spectra of sample and std solns from 2 to 15 μ and compare for identity of sample.

MYDRIATICS AND MYOTICS

Cat-Eye Bioassay Method (138)—Official

32.264 APPARATUS

(a) *Mohr pipets.*—1 ml, graduated in 0.1 ml, with slender tips that deliver exactly 0.05 ml /drop.

(b) *Nitrogen-filled electric lamps.*—100-watt or equally intense illumination.

32.265 ANIMALS

Adult cats.—In good physical condition, weighing >1500 g, and accustomed to being handled.

32.266 PREPARATION OF SAMPLE

Dissolve, in ca neutral H_2O, representative number of tablets, or enough powder, to make soln contg 1 mg alkaloid/ml. If alkaloids themselves are taken, add equiv. quantities of acid to convert them to corresponding salts. Add 2 drops ca 0.02*N* acid/50 ml soln.

For greater accuracy, results of chemical assay upon sample should be followed in prepn of solns; when such accuracy is unnecessary, declaration of concn on label may be accepted as basis for prepn of soln.

One drop of respective concns of following drugs is min. effective dose:

MYDRIATICS

	mg/L
Atropine	12
Hyoscyamine	4
Scopolamine	0.4
Homatropine	200
Cocaine	60
Euphthalmin (Eucatropine)	50,000
Ephedrine (alkaloid)	2,500

Ephedrine salt (or synthetic ephedrine)	50,000
Pseudoephedrine (alkaloid)	2,500
Pseudoephedrine (salt)	80,000
MYOTICS	
Pilocarpine	25,000
Physostigmine (eserine)	10
Arecoline	10,000

32.267 DETERMINATION OF CAT'S THRESHOLD

Place cat ca 1 foot from 100-watt elec. lamp, and det. max. contractility of its pupils under this condition. Drop 0.05 ml of the freshly prepd std mydriatic soln, obtained by dilg the 1 mg/ml soln, into outer margin of one eye, leaving other eye untreated as control. Compress inner canthus, while opening and closing lids, until fluid has apparently disappeared (10–30 sec.). Return cat to cage.

One and 2 hr after application (for atropine, 3 and 4 hr also), place cat under same conditions, and note any differences in diam. between pupils of treated and untreated eyes. (Satisfactory reaction is produced when pupil of treated eye is just perceptibly wider (0.5–1.0 mm) than pupil of untreated eye.) Do not use same eye for another assay for at least 24 hr.

If concns given fail to produce satisfactory reaction, repeat test with more or less concd soln until min. effective concn is found. (This concn may vary somewhat for different cats, but it is essentially constant for same cat.)

32.268 BIOASSAY OF UNKNOWN SOLUTIONS

Dil. the 1 mg/ml soln to be tested to min. effective concn for cats to be used, and drop 0.05 ml of this diln into one eye of the cat, following same procedure as in detn of min. effective concn. Also prep. more or less concd solns and apply to one eye of each of other cats used. Test various concns until one is obtained that produces satisfactory mydriasis of same degree as std soln when tested on 2 or more cats.

To obtain mg alkaloid present in each ml original soln, multiply mg/ml found to be cat's min. effective concn by diln used. Knowing that original soln was made to contain 1 mg alkaloid/ml, calc. quantity of mydriatic present, and express as % total alkaloid.

PHENOLIC DRUGS

Dinitrophenol (or its Sodium Compound) (*139*)—Official

32.269 REAGENTS

(**a**) *Potassium iodide soln.*—Dissolve 20 g KI in 100 ml H_2O.

(**b**) *Bromide-bromate soln.*—0.1*N*. Dissolve 2.7836 g $KBrO_3$ and 12 g KBr in H_2O and dil. to 1 L. If necessary, stdze against 0.1*N* $Na_2S_2O_3$ as in **32.128.**

32.270 DETERMINATION

(**a**) *Interfering substances absent.*—Weigh 0.18–0.20 g 2,4-dinitrophenol (or enough prepn to contain that quantity) into 100 ml beaker, and dissolve in 25 ml H_2O, using enough 2% NaOH soln to insure soln. Transfer soln to 500 ml g-s. flask, using H_2O for washing (do not use heat). Dil. with H_2O to ca 100 ml, and add 25 ml of the KBr-$KBrO_3$ soln and 10 ml HCl. Immediately stopper flask and swirl vigorously 1–3 min. Remove stopper and quickly add 5 ml of the KI soln, taking care to avoid loss of Br; immediately stopper flask and shake thoroly ca 1 min. Remove stopper and rinse down neck of flask with H_2O. Titr. with 0.1*N* $Na_2S_2O_3$ soln, **32.096(a)**, using starch indicator, **4.004(f)**, near end point. 1 ml 0.1*N* KBr-$KBrO_3$ = 0.0092 g 2,4-dinitrophenol; 0.0103 g Na dinitrophenate; 0.0112 g Na dinitrophenate monohydrate.

(**b**) *Interfering substances present.*—Weigh into separator sample contg ca 0.18 g 2,4-dinitrophenol or its Na compound. Macerate short time with 10 ml H_2O and 10 ml 2% NaOH soln. Acidify with HCl. Ext. with 10–20 ml $CHCl_3$ and repeat until extn is complete (usually 5 or 6 extns are necessary), avoiding vigorous shaking, particularly during first 2 extns. Test for complete extn by shaking last $CHCl_3$ ext. with 5 ml 2% NaOH soln (yellow color in latter indicates incomplete extn; 5 ml contg 0.025 mg is pale yellow).

Combine $CHCl_3$ exts in separator and shake with 10–15 ml 2% NaOH soln. Drain $CHCl_3$ layer into third separator and repeat extn until no more yellow color is extd. Note total vol. NaOH soln used. Transfer alk. solns to 500 ml g-s. flask, washing separator each time with H_2O. Add exact quantity of HCl necessary, previously detd, to neutralize NaOH soln used. Proceed as in (**a**).

Guaiacol (*140*)—Official

32.271 REAGENT

Hydriodic acid.—Sp. gr. 1.7. Boil HI 30 min. under reflux with excess of hypophosphorous acid. When cool, transfer to dark, g-s. bottle. Do not leave bottle unstoppered more than few min.

32.272 APPARATUS

Methoxyl apparatus.—*See* Fig. 85, **38.028.**

32.273 DETERMINATION

Place aliquot of alk. guaiacol soln (guaiacol dissolved in 1% NaOH) contg 0.03–0.06 g guaiacol in boiling flask and evap. soln just to dryness on steam bath in air current. For solid guaiacol compounds, weigh 0.06–0.1 g and transfer directly to flask. Complete detn by method for methoxyl

group, **38.029,** beginning "Add 2.5 ml melted phenol from wide-tip pipet . . . " Boil 30 min. and use 0.1N $Na_2S_2O_3$ for titrn. 1 ml 0.1N I = 0.00207 g guaiacol; 0.00228 g guaiacol carbonate; 0.00404 g K guaiacol sulfonate.

Hexylresorcinol (*141*)—Official

32.274 REAGENTS

(**a**) *Sodium thiosulfate std soln.*—0.1N. *See* **32.096**(**a**).

(**b**) *Purified methanol.*—Purify if necessary as follows: Add enough Br to commercial MeOH to give bright yellow color and heat to boiling on H_2O bath 5 min. Cool, and carefully decolorize by adding 10% $NaHSO_3$ soln dropwise until just colorless.

32.275 STANDARDIZATION OF THIOSULFATE

Add 30 ml 0.1N KBr-$KBrO_3$, **32.128,** and 10 ml purified MeOH to 150 ml g-s. flask. Wet stopper. Add 5 ml HCl, stopper flask, immediately place under running tap H_2O, and swirl until flask cools to room temp. Continue to shake flask 5 min. after adding HCl. Cautiously loosen stopper and add 5 ml KI soln, **32.269**(**a**). Swirl gently to liberate the I, wash stopper, and titr. with the $Na_2S_2O_3$ soln. Add starch paste when color of soln is pale yellow.

32.276 DETERMINATION

Transfer 0.07–0.09 g sample to 150 ml g-s. flask Add 10 ml of the MeOH and swirl gently to dissolve sample. Add 30 ml of the 0.1N KBr-$KBrO_3$. Moisten stopper, add 5 ml HCl, stopper flask, and immediately hold under running H_2O while swirling rather vigorously. When cooled to room temp. (ca 1 min.), remove from tap and continue to shake vigorously 5 min. after adding HCl. Cautiously loosen stopper and add 5 ml KI soln, **32.269**(**a**). Swirl gently, wash stopper with little H_2O, add 1 ml $CHCl_3$, and titr. with $Na_2S_2O_3$ soln while swirling flask gently. Near end point, stopper flask and shake vigorously to get free halogen out of $CHCl_3$. When color becomes pale yellow, add starch paste and continue titrn. End point is reached when starch-I color does not return during 30 sec. of vigorous shaking. 1 ml 0.1N KBr-$KBrO_3$ soln = 0.00488 g hexylresorcinol.

8-Hydroxyquinoline Sulfate (Oxyquinoline) (*142*)—First Action

Method I.

(For quantities of hydroxyquinoline sulfate between 25 and 250 mg. Use this method whenever nature of sample permits.)

32.277 EXTRACTION

(**a**) *Interfering substances absent.*—Dissolve sample in ca 75 ml H_2O and add 5 ml HCl.

(**b**) *Non-oily preparations.*—Ext. preferably from soln alk. with $NaHCO_3$ or borax. If extn from such medium is impracticable, or if compounds of NH_3 or heavy or alk. earth metals are present, add Me red, **42.012**(**a**), and adjust with NaOH and/or HCl to bare acidity. Add NaOAc $.3H_2O$ in proportion of 1 g/100 ml soln. If heavy or alk. éarth metals are present, also add 2 ml HOAc/100 ml soln.

Ext. soln, adjusted by any of these procedures, with enough 20 ml portions $CHCl_3$. For alk. or barely acid soln, usually 6 extns suffice; when extra HOAc was added, 10–12 extns are needed. Test for complete extn by adding little HCl (1+9) to last portion, evapg $CHCl_3$ on steam bath, adjusting to 70°, and adding drop of 0.01N KBr-$KBrO_3$ and then drop of the Me red, which should be bleached immediately.

Ext. combined $CHCl_3$ exts with five 10 ml portions of HCl (1+9). If salicylic acid, volatile oils, etc., are present, wash each portion of acid with same 10 ml ether. If sample contains phenol or other volatile interfering substances, and these have not been completely removed by preceding process, boil acid soln to remove them, keeping vol. ca constant by adding more H_2O.

(**c**) *Ointments, etc.*—Transfer sample to separator with 50 ml ether, and ext. with five 10 ml portions HCl (1+9). If salicylic acid, etc., is present, wash each acid portion with same 10 ml ether. Add the Me red; make just alk. with 10% NaOH soln, then just acid with dil. HCl, and proceed as in (**b**), beginning "Add NaOAc $.3H_2O$ . . . "

32.278 DETERMINATION

Adjust acid soln obtained in (**a**), (**b**), or (**c**) to 50° and keep at this temp. during titrn by reheating occasionally. Add drop (or more) of the Me red, **42.012**(**a**), from buret and titr. with 0.1N KBr-$KBrO_3$, **32.128.** (Color of liquid gradually changes from brown-orange to yellow; add more indicator whenever yellow color is reached. At slightly beyond half-way point, dibromohydroxyquinoline may crystallize out and adsorb dye. Disregard color of ppt and judge by that of soln. By dilg to not >0.1 g hydroxyquinoline sulfate /100 ml, formation of ppt can be avoided.) End point is reached when, after waiting 10 sec. for absorption of last drop of KBr-$KBrO_3$ soln and adding drop of indicator, it is bleached almost immediately. Timing for addn of drop of indicator at end point is important, as proper conditions prevail only brief period.

Read vols of 2 solns consumed. Measure 10 ml of the Me red into erlenmeyer, add 2 ml HCl, and titr. with the 0.1N KBr-$KBrO_3$. Correct main titrn for quantity of Br consumed by measured vol. indicator used in titrn. 1 ml 0.1N KBr-$KBrO_3$ = 0.00508 g $(C_9H_7NO)_2.H_2SO_4.H_2O$.

32.279 *Method II.*

(For quantities between 2 and 10 mg)

Ext. as in **32.277.** Start titrn as in **32.278,** using 0.01N KBr-$KBrO_3$, and dild Me red (1 vol. Me red, **32.023(b)**, 4 vols H_2O, and enough NaOH to dissolve dye) instead of stronger reagents. Use as little indicator as possible. When near end point, shown by more rapid consumption of indicator, heat to 70°, and complete titrn at this temp.

32.280 **Phenolsulfonates** (*143*) **—Official**

Dissolve sample contg ca 0.8 g phenolsulfonate in ca 30 ml H_2O and add 5 ml HCl. Titr. with 0.4N KBr-$KBrO_3$ (11.134 g $KBrO_3$+50 g KBr dild to 1 L with H_2O, stdzd against 0.1N $Na_2S_2O_3$). Br is absorbed very rapidly at first, but as titrn proceeds, absorption becomes slower and slower. Titr. as far as possible with no indicator other than fading of Br yellow (usually within 1–4 ml of end point). Then use 0.1% aq. Me orange dropwise, adding no new indicator until previous drop has practically faded. After adding reagent, wait long enough for absorption of Br before adding more Me orange (10 sec. at first; 15 sec. at end of titrn), because in presence of dibromophenolsulfonic acid action of Br on Me orange is much slower than normal. End point is reached when, after waiting 15 sec. for absorption of last drop of Br and adding drop of Me orange, latter fades very appreciably in 10 sec. It is always best, after the Me orange has faded, to add another drop to be sure that first drop was not added too soon. 1 ml 0.4N KBr-$KBrO_3$=0.0232 g Na phenolsulfonate.

Thymol (*144*)**—Official**

32.281 PREPARATION OF SOLUTION

Weigh 2 g pulverized thymol, transfer to 500 ml vol. flask, and add 25 ml 25% NaOH soln. Agitate until thymol is dissolved and dil. to mark at 20° with H_2O.

DETERMINATION

32.282 *Method I.*

Transfer 25 ml aliquot of the thymol soln to 250 ml g-s. erlenmeyer, add 20 ml hot HCl (1+1), and immediately run in 1–3 ml less than theoretical quantity of 0.1N KBr-$KBrO_3$, **32.128.** Warm to 70–80°, add 2 drops 0.1% aq. Me orange, and titr. slowly with the KBr-$KBrO_3$ soln, swirling vigorously after each addn. When red color of the Me orange is bleached, add 2 drops of the titrg soln, stopper, shake vigorously 10 sec., add drop of the Me orange, and again shake vigorously 10 sec. Continue addn of the KBr-$KBrO_3$ soln, 2 drops at time, shaking each time until red color disappears. Add 1 drop of the Me orange, shake vigorously, and if red color does not disappear, repeat alternate addn of 2 drops of the KBr-$KBrO_3$ soln and 1 drop of the Me orange, shaking after each addn as directed previously until red color disappears. Calc. ml KBr-$KBrO_3$ soln used to % thymol. 1 ml 0.1N KBr-$KBrO_3$ =0.003753 g thymol. Reserve mixt. in titrg flask for **32.283.**

32.283 *Method II.*

To cooled mixt. from titrn, **32.282,** add 3–5 ml addnl KBr-$KBrO_3$ soln. (If sample has not been previously analyzed by **32.282,** approx. quantity of KBr-$KBrO_3$ soln to use may be detd by adding 20 ml HCl (1+1) to 25 ml soln, **32.281,** heating to ca 80°, and titrg slowly with the KBr-$KBrO_3$ soln, while vigorously swirling flask, to yellow color, permanent 1 min.) Stopper, shake, add 1 g solid KI, wash sides of flask and stopper with H_2O, and titr. I liberated by the excess KBr-$KBrO_3$ soln with 0.1N $Na_2S_2O_3$, using starch soln, **4.004(f)**, as indicator. Calc. quantity of $Na_2S_2O_3$ soln used in terms of the KBr-$KBrO_3$ soln, deduct from total quantity of KBr-$KBrO_3$ soln added, and calc. to % thymol.

32.284 **Thymol in Antiseptics** (*145*)**—Official**

If alc. content is not known, make preliminary alcohol detn.

Transfer 50 ml sample (or aliquot contg 0.05–0.10 g thymol) to Pt or porcelain evapg dish. Add 6–7 ml 50% NaOH soln, mix well, and carefully dealcoholize by placing dish on steam bath before elec. fan. Evap. vol. slightly more than quantity of alcohol present. (If >30% alcohol is present, dil. with H_2O to alc. content of 25%. In no case should evapn be carried beyond 70% of original vol.) Transfer soln to 125 ml separator, washing out evapg dish with enough H_2O to bring vol. to ca 75 ml.

Ext. alk. soln with two 20 ml portions petr. ether. Wash combined exts once with 5–10 ml 5% NaOH soln and add washings to aq. layer. Ext. aq. soln contg the thymol together with Na salts of boric, benzoic, and salicylic acids, with ether as in **32.001,** using 20, 15, 15, 10, and 10 ml. Use 8–10 extns if prepn contains glycerol. Combine ether exts, transfer to 250 ml g-s. erlenmeyer, add 5 ml recently prepd alc. KOH soln, **26.022,** and evap. most of ether, using steam bath and elec. fan. Do not evap. entirely to dryness. Leave 6–8 ml residue and add to it 75 ml hot H_2O (80–90°) and 10 ml HCl.

Immediately run in 1–3 ml less than theoretical quantity of 0.1N KBr-$KBrO_3$, **32.128,** swirling constantly. Add 2 drops aq. 0.1% Me orange and titr. slowly with the KBr-$KBrO_3$ soln, shaking vigorously after each addn. When red color of Me orange is bleached, add 2 drops of the titrg soln, stopper, shake vigorously 10 sec., add 1 drop of

the Me orange, and again shake vigorously 10 sec. Continue addn of the KBr-$KBrO_3$ soln, 2 drops at time, shaking after each addn until red color disappears. Add 1 drop of the Me orange, shake vigorously, and if red color does not disappear, repeat alternate addn of 2 drops of the KBr-$KBrO_3$ soln and 1 drop of the Me orange, shaking after each addn as above, until red color disappears. 1 ml 0.1*N* KBr-$KBrO_3$ = 0.003753 g thymol.

Test for complete extn by shaking aq. layer with two 15–20 ml portions ether and titrg the thymol, if any, in the ether exts. Add this titrn to that obtained for main ether ext.

If theoretical quantity of thymol present is not known, add 2 drops Me orange and titr. slowly, swirling constantly during addn of KBr-$KBrO_3$ soln until red color is bleached. Continue as above, beginning "add 2 drops of the titrg soln, stopper, shake vigorously . . . "

CAUTION: To avoid loss of thymol by volatilization, both evapn of alcohol and later evapn of ether must be done carefully.

SULFONAMIDE DRUGS

Sulfadiazine and Sulfamerazine (*146*)—First Action

32.285 REAGENTS

(a) *Citrate buffer soln.*—Dissolve 37 g $Na_3C_6H_5O_7.2H_2O$ in H_2O, add 32 ml HCl, and dil. to 250 ml with H_2O.

(b) *2-Thiobarbituric acid soln.*—Recrystallize acid twice from H_2O. Dissolve 5 g recrystd acid in 20 ml 1*N* NaOH dild with 500 ml H_2O. Add 250 ml of the citrate buffer soln and adjust to pH 2.0. Reagent is stable when stored in g-s. bottle in refrigerator.

32.286 DETERMINATIONS

(a) *Sulfadiazine.*—To powd. sample contg ca 0.1 g mixed sulfonamides add 50 ml 1*N* HCl. Shake intermittently 10 min., filter if necessary, and dil. filtrate and washings to 100 ml with H_2O. To 5 ml aliquot add 7.5 ml 1*N* HCl and dil. to 100 ml. Designate this soln (contg ca 5 mg mixed sulfonamides/100 ml 0.1*N* HCl) as Soln A. To 1.0 ml aliquot Soln A in g-s. test tube add 10.0 ml 2-thiobarbituric acid soln, stopper, and heat 1 hr at 100°. Weigh tube before and after heating, and compensate for any loss of moisture by addn of H_2O. Treat similarly 1.0 ml std contg 25 mmg sulfadiazine in 0.1*N* HCl and blank contg 0.1*N* HCl. Det. absorbance of sample, A, and of std, A', at 532 mμ relative to blank.

Mg sulfadiazine in sample taken = 50 A/A'.

(b) *Sulfamerazine.*—Det. absorbance, A_T, of Soln A, and absorbances, A_D', and A_M', of solns contg 5.0 mg pure sulfadiazine and sulfamerazine, resp., in 100 ml 0.1*N* HCl, at 305 mμ relative to 0.1*N* HCl blank. Then absorbance of Soln A due to sulfadiazine $(A_D) = A_D' \times$(mg sulfadiazine in Soln A/5.0), and absorbance due to sulfamerazine $(A_M) = A_T - A_D$.

Mg sulfamerazine in sample taken = 100 A_M/A_M'.

32.287 Sulfadiazine in Presence of Other Sulfonamides (*146*)—First Action

Det. sulfadiazine as in **32.286(a)** from soln prepd to contain ca 25 mmg sulfadiazine/ml 0.1*N* HCl.

32.288 Sulfanilamide (*147*)—Official

Place on 9 cm folded filter paper in funnel portion of sample contg ca 0.5 g sulfanilamide. Wash sol. portion into 250 ml flask with fine stream of acetone, using total of ca 25 ml. Test for complete extn by evapg small portion of washings. Immerse flask in H_2O bath at ca 70° until acetone odor is no longer perceptible. Remove from bath and add 10–12 ml H_2SO_4 (3+1). Connect flask to reflux condenser with H_2O jacket, add few glass beads, and boil soln slowly 30 min. Wash down condenser with H_2O, dil. liquid in flask to ca 100 ml with H_2O, add excess of 50% alkali, distill, and collect NH_3 in distillate in excess of 0.1*N* H_2SO_4. Titr. excess acid with 0.1*N* NaOH, using Me red. 1 ml 0.1*N* H_2SO_4 = 0.01722 g $(NH_2)_2C_6H_4SO_2$.

SYNTHETIC DRUGS NOT OTHERWISE CLASSIFIED

p-Aminosalicylic Acid (PAS) and Isonicotinylhydrazine (INH) (*148*)—Official

32.289 REAGENTS

(a) *Benzaldehyde.*—N.F. or reagent grade.

(b) *Concentrated pH 7 buffer.*—Dissolve 34 g anhyd. KH_2PO_4 in 136 ml 1*N* NaOH and dil. to 1 L with H_2O.

32.290 EXTRACTION OF TABLETS

(a) Weigh accurately sample of powd. tablets contg 35–40 mg INH and transfer to 150 ml beaker. Stir with 20 ml H_2O, add 1.5 g $NaHCO_3$, and continue stirring until effervescence stops. Filter with suction thru medium porosity fritted glass filter (3.5 cm diam. is convenient) precoated with ca 3 mm layer of Celite No. 545. Rinse beaker thoroly with 5 ml H_2O, break suction, transfer rinsings to funnel, washing down inside wall, and reapply suction. Repeat washing of beaker and funnel with 3 addnl 5 ml portions H_2O. Quantitatively transfer filtrate to 50 ml vol. flask with aid of small portions of H_2O, dil. to vol., and mix. Proceed immediately with detn of PAS. Det. INH as soon as practicable, preferably not >4 hr after prepn of $NaHCO_3$ soln.

(b) (*Applicable when filtration with suction is*

not feasible).—Weigh sample as in (a), and transfer quantitatively to 40–50 ml round-bottom centrifuge tube. Add cautiously, in small portions, freshly prepd soln of 1.5 g $NaHCO_3$ in 20 ml H_2O. Agitate mixt. well after each addn, avoiding loss from foaming by adding few drops of ether from time to time. After all $NaHCO_3$ soln is added, continue agitation until effervescence stops. Centrifuge 5–10 min. at ca 2000 rpm and decant supernatant into 50 ml vol. flask. Add 10 ml H_2O to tube, using rubber policeman to wash down wall, to disintegrate residual cake, and to secure uniform suspension. Centrifuge as before, and combine supernatant wash with original ext. Repeat washing with three 5 ml portions H_2O, dil. combined aq. phases to vol., mix, and filter thru fluted paper. Proceed as in (a).

32.291 p-aminosalicylic acid (pas)

From aq. $NaHCO_3$ ext., transfer aliquot contg ca 150 mg PAS to 500 ml vol. flask and dil. to mark with H_2O. Transfer 10 ml aliquot to 250 ml vol. flask, add 12.5 ml concd pH 7 buffer, and dil. to vol. with H_2O. With Beckman Model DU spectrophotometer (or equiv.) measure absorbance of this diln in 1 cm cell at 299 (max.), 244 (min.), and 325 mμ against 1+19 diln of the buffer. (With instruments suitable for absorbance readings in range 1.0–1.5, use 2 cm cell thruout method or modify diln so that concn of substance is twice that specified.) Calc. baseline absorbance: $A_B = A_{299} - (0.3210\ A_{244} - 0.6790\ A_{325})$.

Accurately weigh ca 50 mg finely powd. pure PAS, dissolve in 2 ml alcohol, add 5 ml 0.1*N* NaOH, and dil. with H_2O to exactly 500 ml. Transfer 25 ml aliquot to 200 ml vol. flask, add 10 ml concd pH 7 buffer, and dil. to vol. with H_2O. Det. absorbance at 244, 299, and 325 mμ as above. Det. A_B, and from this value and that obtained from sample soln, calc. quantity PAS in sample.

32.292 isonicotinylhydrazine (inh)

Transfer 20 ml aliquot of the $NaHCO_3$ ext. to 125 ml separator, add 0.5 ml benzaldehyde, shake 15 min., and let stand 10 min. Ext. with six 20 ml portions $CHCl_3$, filter exts thru compact pledget of absorbent cotton into 150 ml beaker, and evap. filtrate on steam bath in air current until residue has only faint odor of benzaldehyde. Rinse down wall of beaker with little $CHCl_3$ to conc. residue at bottom, and evap. to dryness. Add 1–2 ml $CHCl_3$, evap. again to dryness on steam bath in air current, and heat residue few min. Repeat $CHCl_3$ and heating treatment until hot residue of benzylidine isonicotinylhydrazine (BINH) is odorless, or has at most very faint odor of benzoic acid (there must be no sweet odor or odor of benzaldehyde; take care to avoid loss from spattering).

Dissolve residue in $CHCl_3$ and transfer quantitatively to separator with addnl solvent. Add $CHCl_3$ to vol. of 20–30 ml, shake with 10 ml freshly prepd 5% $NaHCO_3$, and filter $CHCl_3$ layer thru compact pledget of absorbent cotton. Wash aq. soln with three 10 ml portions $CHCl_3$, passing each wash thru filter, and evap. combined $CHCl_3$ exts to dryness on steam bath in air current.

Dissolve residue of BINH in alcohol without heat, and dil. to exactly 100 ml with alcohol. Dil. 5 ml aliquot of this soln to exactly 200 ml with alcohol, and det. absorbance of diln with Beckman Model DU spectrophotometer, or equiv. (1 cm cell; alcohol blank) at 302 (max.) and 375 mμ. Subtract reading at 375 (background absorbance from impurities) from that at 302 mμ. Difference represents absorbance from BINH at 302 mμ.

Dissolve ca 20 mg sample, accurately weighed, of pure BINH in alcohol and dil. to exactly 100 ml. Dil. 10 ml aliquot of this soln to exactly 250 ml with alcohol and det. absorbance at 302 mμ. Using this value and that due to BINH obtained from sample, calc. quantity of BINH in sample. BINH×0.60888 = INH.

Amphetamine (*149*)—First Action

32.293 *Titrimetric Method*

Weigh accurately not <20 tablets and det. av. wt/tablet. Grind tablets in mortar to pass No. 80 sieve. Weigh accurately enough powder to provide 125 mg amphetamine sulfate (or equiv. of other salt), transfer to 100 ml beaker, add 15 ml H_2O, and stir 15 min. Using rod or policeman, transfer as much of suspension as possible to fritted glass funnel (medium porosity, 40 mm disk is convenient) and filter with suction into suitable vessel. Break suction and, with portions of H_2O totaling 15 ml, rinse as much material as possible from beaker into funnel. Triturate mixt. in funnel to uniform paste and reapply suction. Make transfer and filtration quant. by repeating washing with 4 addnl 10 ml portions H_2O. Quantitatively transfer filtrate to 100 ml vol. flask with small portions of H_2O, dil. to vol., and mix.

Transfer 40 ml aliquot to separator, add 1 ml 10% NaOH soln, and ext. with six 25 ml portions ether as in **32.001**. Wash combined ether exts with two 5 ml portions H_2O, and ext. combined washings with two 10 ml portions ether. Combine ether washings with main ether ext., filter thru cotton pledget into 250 ml separator, thoroly rinse separator which contained unfiltered ether exts with ether, and filter these rinsings into filtered ether exts. Ext. filtrate with exactly 20 ml 0.02*N* H_2SO_4 and drain acid ext. into 200 ml

erlenmeyer. Wash ether with 10, 5, and 5 ml portions H_2O, combine washings with acid ext., and heat on steam bath until dissolved ether is expelled. Cool, and titr. soln with $0.02N$ NaOH, using Me red. Calc. % amphetamine sulfate in sample and quantity/tablet. 1 ml $0.02N$ H_2SO_4 =3.685 mg amphetamine sulfate.

32.294 *Confirmatory Gravimetric Determination*

Combine titrd soln, **32.293,** in 250 ml separator with 50 ml aliquot of the 60 ml unused aq. ext. remaining in vol. flask, acidify by dropwise addn of $0.1N$ H_2SO_4, and ext. with three 10 ml portions CCl_4. Discard CCl_4 exts, sepg as much CCl_4 as practicable. Add 4.10 g $NaHCO_3$ to aq. soln, and swirl separator until most of salt dissolves. Add rapidly 1.0 ml Ac_2O by blowing in from 1 ml pipet. Immediately stopper separator securely and shake vigorously until evolution of CO_2 nearly ceases (frequently release pressure during shaking by opening stopcock). Add another 1.0 ml portion Ac_2O, and keep shaking separator until evolution of CO_2 ceases (5–10 min. after addn of second portion of anhydride).

Let mixt. stand 5 min., and completely ext. acetylamphetamine by shaking with 50 ml portions $CHCl_3$ (4 should be enough; test fifth for complete extn). Filter exts thru cotton pledget, rinse filter with $CHCl_3$, evap. filtrate to small vol. on steam bath in current of air, quantitatively transfer soln to tared 50 ml beaker by rinsing with small portions of $CHCl_3$, and continue evapn until solvent is removed. Heat residue of acetylamphetamine in oven (not force-draft type) 1 hr at 80°, cool in desiccator, and weigh. Calc. % amphetamine salt in sample and quantity/tablet. Acetylamphetamine $\times$1.0395 =amphetamine sulfate.

Induce crystn in residue, if it has not crystd spontaneously, by trituration, adding small seed crystal of racemic acetylamphetamine if necessary. Finely powder cryst. derivative, and mix well. Pure acetyl-*d*-amphetamine melts at 124.5–125°; racemic derivative melts at 93–93.5°.

Stereochemical Composition of Amphetamines

32.295 *Polarimetric Method*

Weigh accurately 90 mg acetyl derivative, **32.294,** transfer quantitatively to 5 ml vol. flask, and dil. to vol. with $CHCl_3$. Det. optical rotation of soln in semi-micro 2 dm (200 mm) tube (bore, ca 4.5 mm; vol., 3–4 ml) at same temp. as soln was dild to vol. Acetyl-*d*-amphetamine is levorotatory in $CHCl_3$.

In measuring rotation with polariscope, take 10 readings on soln and calc. av. to 0.001°. Det. av. reading with same tube filled with $CHCl_3$ similarly, and use av. zero-point reading thus obtained to correct av. reading given by soln. If saccharimeter is used instead of polariscope, estimate all readings to 0.05 division, calc. av. to 0.01 division, correct for zero-point, and multiply value so obtained by appropriate factor, **29.020**(**c**), or by 0.3462 (°S) to obtain rotation, α, in angular degrees.

Calc. specific rotation, $[\alpha]$, to 0.1° by equation $[\alpha]=100\alpha/c\times l$, in which c=acetylamphetamine concn in g/100 ml and l=tube length in decimeters.

Det. % *d*-amphetamine by equation: $\% \ d=50+(50[\alpha]/44)$, in which $[\alpha]$=specific rotation of acetyl derivative from sample, and 44=specific rotation of pure acetyl-*d*-amphetamine; sign of rotation is ignored.

32.296 *Confirmatory Thermal Analysis*

In m. p. tube, 2–3 mm i. d. at bottom and ca 70 mm long, place enough finely powd. acetyl-*dl*-amphetamine (ca 8 mg) to form column 5–6 mm high after tube and contents have been tapped firmly several times on hard surface.

Select thermometer with 90–130° range, with graduations permitting readings to 0.5° with aid of low-power hand lens. Anschütz-type thermometer is convenient but not necessary. Thermometer need not be calibrated, but if not, same thermometer must be used in detg std m. p. curve and m. p. of derivative from sample.

Fix m. p. tube securely to thermometer by 2 small rubber bands, one near top of tube and other as far down as possible without letting liquid in bath touch band. (Bands may be cut from rubber tubing of proper size.) Adjust tube so that middle of column of specimen coincides approx. with middle of thermometer bulb.

Support assembly in mechanically-stirred m. p. bath. (*DC 200 Silicone fluid,* viscosity grade 20 centistokes at 25°, made by Dow Corning Corp., Midland, Mich., is convenient bath liquid.) Raise temp. of bath rapidly until it is ca 5° below anticipated m. p. (temp. at which specimen becomes entirely clear liquid); then regulate heating carefully so that rise in temp. is not $>0.5°$/min. After specimen begins to melt, stir continuously with chromel wire (0.4 mm diam.; flatten ca 3 mm of lower end and bend flattened portion at right angle ca 1 mm from tip so as to form hoe-like stirrer) while inspecting it carefully with ca 10$\times$ hand lens. (Observation is facilitated by passing beam of light thru specimen from rear.) Note temp. at which last crystal disappears, and record this as m.p. Remove tube and thermometer from bath, induce melt to solidify by stirring (seeding if necessary), and repeat detn. Replicate detns will not differ by $>1°$ if carefully per-

formed. Following same procedure, det. m.p. of pure acetyl-*d*-amphetamine.

Prep. series of std mixts of acetyl-*d*- and acetyl-*l*-amphetamine with following compositions, expressed in mg: $80d+20dl$, $60d+40dl$, $40d+60dl$, and $20d+80dl$. These mixts contain, resp., 90%, 80%, 70%, and 60% *d*-isomer. In each case accurately weigh each component into small (18×55 mm) test tube, hold tube in bath heated to 130–135° until contents melt completely, stir molten contents with small stainless steel spatula until well mixed, and then withdraw tube from bath and continue to stir until melt solidifies completely. Transfer solidified material as completely as possible to small mortar, powder finely, and mix thoroly. Det. m. p. of each mixt. as above. In each mixt. beginning of fusion (softening, appearance of liquid phase) will be noted at ca same temp. (ca 93°), but temp. at which system becomes entirely liquid (m.p.) will depend on composition of mixt. Unlike m.p. detns of pure *dl*- and *d*-derivatives, it is not important to stir mixts continuously after first evidence of fusion. After considerable liquid phase forms, stir sample occasionally as solid phase diminishes. Stir continuously during ca last 2 min. of detn, *i.e.*, during inspection in anticipation of disappearance of last portion of cryst. matter. Push down any solids adhering to walls of tube above melt into melt with wire stirrer.

Plot on coordinate paper av. m.p. (ordinate) of each specimen against composition (abscissa) expressed as % acetyl-*d*-amphetamine, and draw best smooth curve thru the 6 plotted points.

Det. m.p. of derivative obtained from sample and estimate % *d*-isomer present from std curve.

32.297 Chlorobutanol *(150)*—**Official**

(**a**) *Chlorobutanol crystals.*—Transfer to pressure bottle sample contg ca 0.3 g chlorobutanol and carefully add 25 ml alc. KOH soln, **32.298(a)**. Stopper bottle; swirl gently, taking care to prevent soln from contacting rubber washer; then let stand 30 min. or overnight. Place bottle in wire basket, and set basket in H_2O bath at room temp. Invert tin can over bottle and cover with towel to prevent injury in case bottle should burst. Heat bath to boiling and maintain this temp. 15 min. Cool gradually.

Add 25 ml H_2O, swirling gently, and transfer contents of pressure bottle to 400 ml beaker. Wash bottle with H_2O, draining washings into beaker. Add 15 ml HNO_3 and excess of 2% $AgNO_3$ soln, stir well, and let mixt. stand in dark 15 min. Collect ppt in gooch previously dried at 105° and weighed. Wash ppt thoroly with H_2O and then with 5 ml alcohol followed by 5 ml ether. Dry to constant wt at 105°. If reagents contain Cl, apply correction detd by blank test. 1 g AgCl = 0.4127 g $C_4H_7OCl_3$.

(**b**) *Aqueous ampul solns.*—Pipet into distg flask sample contg ca 0.1 g chlorobutanol. Add enough H_2O to bring vol. to 50 ml and distill ca 25 ml thru straight-bore condenser. Collect distillate in ca 100 ml pressure bottle contg 25 ml alc. KOH soln, **32.298(a)**, and surrounded by ice bath. Have delivery tube extend into alc. soln. (Use straight-bore condenser to assure complete soln of crystals of chlorobutanol in condenser.) Let cool, disconnect still head, and wash condenser carefully with 25 ml alcohol, letting alcohol drain into pressure bottle. Repeat washings, using ca 20 ml H_2O. Also wash receiving tube with H_2O. Stopper bottle and swirl gently, taking care to prevent soln from contacting rubber washer. Let stand 30 min. or overnight. Complete detn of Cl as in (**a**).

Chloroform or Carbon Tetrachloride *(151)*—Official

32.298 REAGENTS

(**a**) *Alcoholic potassium hydroxide soln.*—Dissolve 35 g KOH (Cl-free) in MeOH to make 100 ml. Let stand several days and decant clear liquid.

(**b**) *Ammonium thiocyanate soln.*—0.05*N*. Adjust by titrg against 0.1*N* $AgNO_3$.

(**c**) *Ferric ammonium sulfate indicator.*—Dissolve 8 g $FeNH_4(SO_4)_2.12H_2O$ in enough H_2O to make 100 ml.

32.299 WEIGHING OF SAMPLE

(**a**) *Chloroform or carbon tetrachloride.*—Carefully transfer 30 ml of the alc. KOH soln to air-dried, 60–70 ml pressure bottle, and stopper. Do not moisten neck of bottle with the reagent. Weigh stoppered bottle with contents (conveniently done by suspending bottle on balance with clamp that holds stopper).

Immediately after opening bottle, add ca 1 ml sample from 1 ml pipet, holding pipet just above top level of reagent in bottle. As level of reagent rises with draining of sample into bottle, raise pipet correspondingly so as to avoid contact with reagent. Avoid opening bottle longer than necessary (20 sec. is convenient time). Stopper bottle so as to assure tight fit and weigh. Det. wt by difference. Proceed as in **32.300**.

(**b**) *Carbon tetrachloride in capsules.*—Det. gross wt of representative number of capsules. Open capsules and transfer contents to suitable flask. Weigh dried empty capsules and det. av. net contents. Proceed as in (**a**), using composite sample.

(**c**) *Chloroform or carbon tetrachloride in drug mixtures.*—Proceed as in (**a**), using not >10 ml

of the mixt. contg 0.08–1.6 g $CHCl_3$ or CCl_4. Note temp. of mixts. Det. vol.-equiv. of weighed sample. (Weigh definite vol. of mixt. at same temp., using 50 or 100 ml vol. flask, and calc.)

NOTE: Sample may be measured directly with pipet instead of being weighed, or measured vol. may be dild with MeOH to some definite vol. and thoroly mixed, and suitable aliquot of this diln used.

32.300 DETERMINATION

If sample is mixt., mix contents of bottle by gentle swirling and let bottle stand ca 1 hr (30 min. is enough for $CHCl_3$, pure or nearly so). Place bottle in wire basket and set basket in H_2O bath at room temp. Invert tin can over bottle and cover with towel to prevent injury in case bottle should burst. Heat bath to boiling and keep at this temp. 1 hr (15 min. is enough for $CHCl_3$, pure or nearly so). Cool contents of pressure bottle gradually, transfer to 200 ml vol. flask, and wash out bottle thoroly with H_2O, draining washings into flask. Bring to room temp., fill to mark with H_2O, and mix.

Transfer suitable aliquot to 100 ml vol. flask and acidify with HNO_3, adding ca 2 ml excess. Add 25 or 50 ml 0.1*N* $AgNO_3$ (an excess), shake thoroly, dil. to mark with H_2O, and mix. Filter mixt. thru dry filter into dry flask, rejecting first 20 ml filtrate. To 50 ml aliquot filtrate, add 3 ml of the $FeNH_4(SO_4)_2$ indicator and titr. excess $AgNO_3$, using 0.05*N* NH_4 or K thiocyanate.

If original sample contains Cl, det. quantity and make correction. If original sample contains sugar or other org. material and (after saponification of the $CHCl_3$ or CCl_4 and diln of mixt. with H_2O) is highly colored, thus interfering with titrn, transfer contents of pressure bottle to Ni crucible with H_2O. Evap. to dryness and char residue. Let cool, treat with H_2O, filter into suitable vol. flask, and wash residue and filter with H_2O until free from Cl. Dil. to mark with H_2O, mix, and det. Cl as directed previously.

Make blank test, using in pressure bottle same quantities of solvents and reagents as for sample, and apply necessary correction. 1 ml 0.1*N* $AgNO_3$ $=0.00398$ g $CHCl_3$ or 0.00385 g CCl_4.

Ether (*152*)—Official

(Not applicable in presence of essential oils)

32.301 REAGENTS

(**a**) *Sulfuric acid.*—(1+1). Carefully add H_2SO_4 to equal vol. H_2O and cool to room temp.

(**b**) *Potassium dichromate soln.*—1*N*. Dissolve 49.037 g pure $K_2Cr_2O_7$ (or corresponding quantity of known purity) in enough H_2O to make 1 L.

(**c**) *Sulfuric acid-potassium dichromate soln.*—0.5*N*. Carefully add 500 ml H_2SO_4 to 500 ml of the 1*N* $K_2Cr_2O_7$ (accurately measured in vol. flask), and cool to room temp. Use two 1 L flasks for mixing and cooling. Transfer to 1 L vol. flask, using H_2SO_4 (1+1) for washing, and dil. to mark with H_2SO_4 (1+1). Mix thoroly.

Stdze against 0.05*N* $Na_2S_2O_3$ soln as follows: Pipet exactly 25 ml Reagent (**c**) into 250 ml g-s. vol. flask and dil. to mark with H_2O after cooling to room temp. Mix thoroly. Pipet 50 ml aliquot into 500 ml g-s. flask; add 100 ml H_2O, 10 ml H_2SO_4, and 10 ml 25% KI soln, freshly prepd. Stopper flask and let stand 3–5 min. Add 150–200 ml H_2O and titr. with 0.05*N* $Na_2S_2O_3$, using freshly prepd starch soln, **4.004(f)**, as indicator.

32.302 APPARATUS

Set up app. illustrated in Fig. 67. Beginning at air intake end of aspiration train, use 400 ml bottle, *A*, as wash bottle; six 50 ml graduated cylinders, 1.5 cm i. d. and 32–35 cm high, *B-C-D-E-F-G*; 500 ml bottle as safety reservoir, *H*; and 400 ml bottle, *I*, as wash bottle, which is supplied with soda-lime tube, *J*. Supply each container with closely fitting rubber stopper and vapor-carrying tubes. Have intake tube extend almost to bottom and have outlet tube 1 cm below rubber stopper. Use heavy-wall glass tubing 5 mm o. d. Draw out-

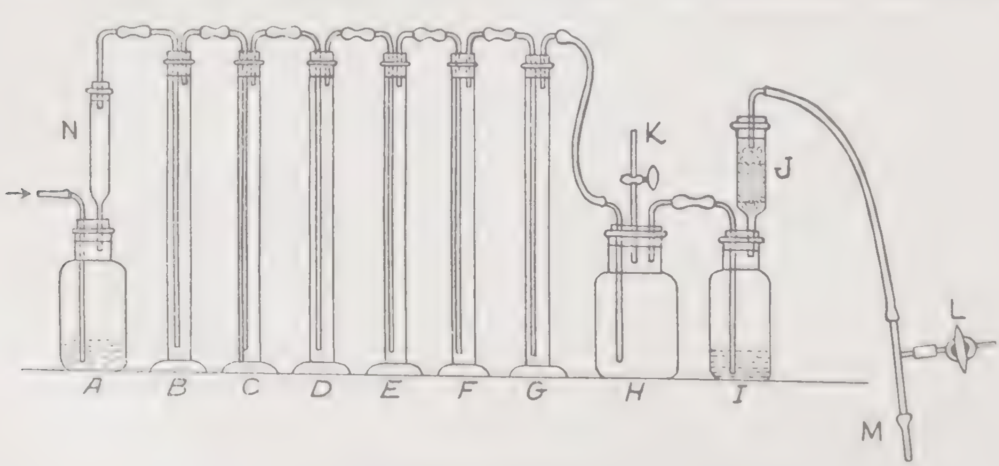

FIG. 67.—APPARATUS FOR DETERMINATION OF ETHER

lets of all vapor-carrying tubes down to small openings. Use heavy-wall rubber tubing for connections and between cylinders expose only 0.5–1 cm to vapors.

32.303 PREPARATION OF SAMPLE

Carefully weigh 100 ml g-s. vol. flask contg 65–70 ml H_2O. Pipet 5 ml ether, holding pipet just above H_2O in flask; as level of H_2O is raised by draining of ether into flask, raise pipet correspondingly to avoid contact with the H_2O. Immediately stopper flask and weigh. Increase in wt is wt ether. Carefully and gently swirl liquid in flask until ether dissolves and then dil. to mark with H_2O. Stopper flask and mix thoroly. If sample is alc. or hydro-alc. ether soln, prep. soln by diln with H_2O to meet requirements in **32.305.**

32.304 PRELIMINARY CHARGING OF APPARATUS

Transfer ca 100 ml of the H_2SO_4-$K_2Cr_2O_7$ soln to wash bottle *A*, and 35 ml of the H_2SO_4 (1+1) to each cylinder, *C* and *D*. (Use funnel with long stem to avoid wetting upper portion of container.) Pipet 40, 25, and 25 ml of the H_2SO_4-$K_2Cr_2O_7$ soln into cylinders *E*, *F*, and *G*, resp., avoiding unnecessary wetting of outside of pipet stem and touching inside of cylinder with the wetted stem of the pipet while draining. Bottle *H* remains empty. Transfer ca 50 ml H_2SO_4 to bottle *I* and fill tube *J* with appropriate quantity of soda-lime, layered on bottom and top with cotton. Stopper tightly all containers except cylinder *B*. Leave open all rubber tubing connections between cylinders and glass stopcocks *K* and *L*.

32.305 DETERMINATION

If sample is known not to contain alcohol or other substances, other than ether, that will be oxidized by the H_2SO_4-$K_2Cr_2O_7$ soln, pipet aliquot of sample soln, **32.303,** into 250 ml g-s. flask contg 50 ml of the H_2SO_4-$K_2Cr_2O_7$ soln. Stopper flask, swirl gently, and let stand 1 hr. Titr. excess acid-$K_2Cr_2O_7$ and calc. as below.

Pipet aliquot of sample contg 0.035–0.2 g ether in aq. soln or hydro-alc. soln, contg not >5 g alcohol, into cylinder *B* contg enough H_2O to make total vol. 25 ml. Hold pipet just above top level of liquid in cylinder, and as liquid is raised by draining of sample, raise pipet correspondingly so as to avoid contact with liquid.

Stopper tightly and immediately connect with cylinder *C* and wash bottle *A*. Connect suction pump at *M*, and with stopcock *L* ca half open, start pump. With bottle *H* and cylinder *G* connected, gradually close stopcock *K* until slow current of bubbles passes thru reagent in cylinder *F*, and connect cylinder *E*. Repeat until cylinder *B*, which contains sample, is connected. Make certain all connections are air-tight. (Usually stopcock *L* requires no further adjustment.) Carefully adjust stopcock *K* until rapid and steady current of bubbles (ca 150/min.) flows thru aspiration train. (Usually this is attained with cock *K* slightly open, depending upon size of opening thru cock *L*.) Use care so no reagent touches rubber stopper by spray or otherwise. As bubbles rise in cylinders *B* and *C* they increase in size and couple up; near surface each bubble occupies entire cross-section of cylinder and has vertical height of 1–1.5 cm.

Aspirate 5 hr. If not certain that all ether has been carried over into the 0.5*N* acid-$K_2Cr_2O_7$ soln, discontinue aspiration as in following paragraph. Transfer contents of cylinder *E* to g-s. 500 ml vol. flask. Pipet 25 ml acid-$K_2Cr_2O_7$ soln into cylinder *E*. Aspirate as before.

Gradually open cock *K* until rate of flow of bubbles is appreciably slower, and disconnect rubber tubing between cylinders *B* and *C*. Gradually open cock *K* as before and disconnect tubing between *C* and *D*. Repeat until all cylinders are disconnected.

Transfer acid-$K_2Cr_2O_7$ soln (contents of cylinders *E*, *F*, and *G*) to g-s. 500 ml vol. flask. Wash cylinders and glass tubes with H_2O and drain washings into flask. Add 200–300 ml H_2O and cool. Add more H_2O and again cool to room temp. Dil. to vol. and mix thoroly. Pipet 25 ml aliquot into 500 ml g-s. flask and continue as in **32.301(c)**, beginning "add 100 ml H_2O . . . " Calc. ml 0.5*N* H_2SO_4-$K_2Cr_2O_7$ soln consumed by sample. 1 ml 0.5*N* acid-$K_2Cr_2O_7$ = 0.00463 g ether.

32.306 **Ethyl Aminobenzoate** (*153*)—**Official**

(a) *In pure drug.*—Accurately weigh 0.12–0.15 g sample into I flask and dissolve in mixt. of 10 ml HCl and ca 200 ml H_2O. Add 0.1*N* KBr-$KBrO_3$, **32.128,** from buret until slight excess is present as shown by light yellow color. Stopper flask, shake, and let stand 5 min. Add 5 ml 20% KI soln, avoiding loss of Br, stopper flask, and shake. Titr. liberated I with 0.1*N* $Na_2S_2O_3$, stdzd as in **32.096(a)**, using starch soln, **4.004(f)**, as indicator. From quantity of KBr-$KBrO_3$ soln used calc. % Et aminobenzoate. 1 ml 0.1*N* KBr-$KBrO_3$ = 0.004127 g Et aminobenzoate.

(b) *In ointments.*—Accurately weigh 2.5–3 g ointment (enough to provide 0.12–0.15 g Et aminobenzoate) in small beaker, dissolve in benzene by warming on steam bath, and transfer to separator, using in all ca 50 ml benzene. Wash beaker with 50 ml HCl (1+19), pour into separator, shake, and transfer aq. layer to second separator. Add 20 ml petr. ether to second separator, shake, and transfer aq. layer to I flask. Ext. benzene soln with 3 addnl 50 ml portions

HCl (1+19), washing each ext. with the petr. ether in second separator and then collecting it in the I flask. Treat combined acid exts as in (a), beginning "Add 0.1N KBr–$KBrO_3$ . . . " and calc. % Et aminobenzoate.

Methapyrilene in Expectorants (*154*)—First Action

32.307 REAGENT

Methapyrilene hydrochloride std soln.—Transfer 60 mg methapyrilene.HCl, accurately weighed, to 200 ml vol. flask. Dissolve in ca 0.1N H_2SO_4 and dil. to vol. with ca 0.1N H_2SO_4. Transfer 5 ml aliquot to 100 ml vol. flask and dil. to vol. with ca 0.1N H_2SO_4. (1 ml = 0.015 mg methapyrilene .HCl.)

32.308 DETERMINATION

Pipet 10 ml sample into separator, make alk. with NH_4OH, and ext. with four 20 ml portions $CHCl_3$. Combine $CHCl_3$ exts in 100 ml vol. flask and dil. to vol. with $CHCl_3$. Transfer aliquot contg 1–3 mg methapyrilene to small beaker and evap. just to dryness on steam bath with air current. Dissolve residue in ca 0.1N H_2SO_4, transfer to 100 ml vol. flask, and dil. to vol. with ca 0.1N H_2SO_4. Det. absorbance of this soln and of std against ca 0.1N H_2SO_4 blank at 315 mμ.

Mg methapyrilene.HCl/100 ml sample = $(A \times 1.5 \times 100 \times 10)/(A' \times \text{vol. aliquot})$, where A = absorbance of sample and A' = absorbance of std.

Norepinephrine (Arterenol) in Preparations of Epinephrine (*155*)—Official

32.309 APPARATUS

(a) *Chromatographic tube.*—Fuse 5 cm length of 5–7 mm (i.d.) tubing to opening in bottom of 25×200 mm test tube.

(b) *Packing rod.*—Approx. 300 mm long. *See* **32.164**(g).

(c) *Hypodermic syringe.*—1 ml without needle, graduated in 0.01 ml.

32.310 REAGENTS

(a) *Packing material.*—Celite 545 (Johns-Manville Co.).

(b) *Glass wool.*—Pyrex No. 800.

(c) *Benzene.*—Distill reagent grade benzene in all-glass app. Shake distillate with H_2O 2–3 min. and filter benzene layer thru paper. Use this H_2O-satd solvent unless dry benzene is specified.

(d) *Concentrated phosphate buffer.*—pH 6. Dil. 50.0 ml 0.2M KH_2PO_4 soln, **13.023**(b), and 5.64 ml 0.2M NaOH, **13.023**(d), to 100 ml with H_2O.

(e) *Norepinephrine std solution.*—Dissolve 19.9 mg *l*-norepinephrine (levarterenol) bitartrate monohydrate in exactly 100 ml H_2O. 1 ml = 0.100 mg norepinephrine base. Discard after 8 hr.

32.311 PREPARATION OF SAMPLE

(a) *Aqueous solns of epinephrine.HCl containing bisulfite and chlorobutanol.*—If soln is 0.1% with respect to "total epinephrine" (epinephrine +norepinephrine), pipet 30 ml sample into 125 ml separator provided with tightly fitting stopper and stopcock. If soln is more concd, use sample contg 30 mg "total epinephrine," and dil. to 30 ml with H_2O.

(b) *Suspensions of epinephrine in oil.*—Mix suspension by gentle swirling and agitation; add to separator accurately measured vol. contg ca 30 mg epinephrine and 25 ml petr. ether, and swirl until oily base dissolves. Add 10 ml 0.05N H_2SO_4 and ext. epinephrine by shaking 1 min. Drain aq. layer into 125 ml separator, and wash petr. ether layer with two 10 ml portions H_2O. Add washes to acid ext., wash combined aq. layers with two 10 ml portions CCl_4, and discard CCl_4. Rinse stopper and mouth of separator with few drops H_2O and let rinsings drain into separator. Proceed as in acetylation, beginning "Add 2.10 g $NaHCO_3$ . . . "

(c) *Ointments of epinephrine bitartrate (petrolatum base).*—Transfer to separator accurately weighed sample contg ca 60 mg epinephrine bitartrate. Add 25 ml benzene and swirl until ointment base dissolves. Proceed as in (b), beginning "Add 10 ml 0.05N H_2SO_4 . . . " except that if bisulfite is present, it must be removed with I as below before proceeding with acetylation.

32.312 ACETYLATION

Add 25 ml CCl_4 and shake vigorously to ext. chlorobutanol. After layers sep. completely, drain and discard solvent, and repeat extn with two 25 ml portions CCl_4. After each extn, drain as much of the solvent as possible. Rinse stopper and mouth of separator with few drops of H_2O, and let rinsings drain into separator. Add 4 drops starch indicator, **32.037**(b); then, while swirling, destroy $NaHSO_3$ by adding *dropwise* I-KI soln, **32.098**(b), until blue color persists. Immediately discharge blue color by adding dropwise 0.1N $Na_2S_2O_3$. Add 2.10 g $NaHCO_3$ (prevent it from contacting wet mouth of separator) and swirl few sec. to dissolve most of the $NaHCO_3$. Immediately, by means of hypodermic syringe, inject rapidly into separator exactly 1 ml Ac_2O (prevent reagent from contacting mouth of funnel). Stopper separator at once and shake vigorously until evolution of CO_2 stops (ca 7–8 min.). Release pressure as necessary by momentarily inverting separator and cautiously opening stopcock.

Let mixt. stand 5 min.; then ext. with six 30 ml portions $CHCl_3$. Filter each ext. thru $CHCl_3$-washed compact pledget of absorbent cotton into beaker, and evap. combined exts to small vol. or to dryness on steam bath in air current. Quanti-

tatively transfer residue with small portions $CHCl_3$ to tared 50 ml beaker and continue evapn until solvent is removed. Dry 30 min. at 105°, let cool in desiccator, and weigh. Wt mixed amorphous triacetyl derivatives of epinephrine and norepinephrine $\times 0.5923 = E =$ "total epinephrine."

32.313 CHROMATOGRAPHIC SEPARATION OF ACETYLATION PRODUCT

Place wad of glass wool in chromatographic tube and compress it tightly at juncture of tube and stem, using the packing rod.

Place 10 g Celite 545 and ca 175 ml benzene in 250 ml beaker. While stirring vigorously and continuously, add 7.0 ml H_2O, drop by drop, to produce uniform solid phase. Transfer to chromatographic tube ca 1/10 of the solid, under benzene, and compress it firmly and evenly with packing rod. While keeping column of benzene above solid in tube, add remainder of solid in beaker in ca 5 equal portions and compress each portion firmly and evenly before adding next. Properly prepd column is ca 65 mm high and permits flow of ca 2–4 ml benzene/min. under head of 8 cm solvent. With wad of absorbent cotton affixed to stiff wire, remove any solid adhering to tube above column. Keep layer of benzene above column until used.

To beaker contg mixt. of triacetyl derivatives add exactly 6 ml *dry* benzene. Warm gently and dissolve residue completely by stirring and swirling. Cover beaker with watch glass to retard evapn, and cool to room temp.

Remove supernatant benzene from tube by careful aspiration, pipet onto top of column accurately measured aliquot of soln of derivative equiv. to 20–25 mg total epinephrine, and immediately place graduated cylinder under tube. As soon as last of benzene soln is absorbed by column, rinse down wall of tube with three 2 ml portions benzene, delivered conveniently from pipet. Let each rinse be completely absorbed before adding next portion. Then carefully add benzene into tube to height of ca 8 cm above top of column, and maintain level of the benzene by employing suitable constant level device. After 160 ml effluent (contg triacetylepinephrine) collects, thoroly rinse tip of tube with $CHCl_3$ and discard effluent and rinsings, or reserve for qualitative tests.

Remove layer of benzene above column by aspiration, place clean receiver under tube, and let $CHCl_3$ pass thru column until 100 ml effluent (contg triacetylnorepinephrine) collects. Evap. effluent to dryness; then transfer to 50 ml beaker, confining residue near bottom of beaker.

32.314 DETERMINATION OF NOREPINEPHRINE

Add exactly 10 ml 0.50*N* HCl to residue of triacetylnorepinephrine, warm gently, and dissolve by stirring and rubbing with rubber policeman. Pour soln into g-s. test tube (15×150 mm is convenient), place tube in boiling H_2O bath, and stopper loosely. After 5 min., stopper tightly and maintain 30 addnl min. at 100°. Remove tube and cool to room temp., lifting stopper slightly from time to time to keep vac. from forming.

Mix contents thoroly and transfer 1 ml aliquot to another g-s. test tube. Neutralize acid by adding exactly 42 mg $NaHCO_3$. (Insure that *all* $NaHCO_3$ is delivered to bottom of tube, and that none adheres to wall above acid layer.) After effervescence stops, add 1.5 ml H_2O and 2.5 ml of the buffer. Mix by swirling, and add 4 drops 0.1*N* I. Swirl, and after exactly 3 min. (timed by stopwatch) from addn of I, add 6 drops 0.1*N* $Na_2S_2O_3$, stopper, and mix thoroly. Measure absorbance at 520 mμ, 3 ± 0.5 min. after addn of the $Na_2S_2O_3$ soln, in 1 cm cells against the pH 6 buffer blank in Beckman Model DU (or equiv.) spectrophotometer.

Transfer 1.5 ml aliquot std soln to g-s. test tube. Add 1 ml H_2O and 2.5 ml concd pH 6 buffer, and swirl. Develop color and measure absorbance at 520 mμ as above.

Calc. amount of norepinephrine in sample originally taken for analysis, and from this value and *E*, calc. % norepinephrine in "total epinephrine."

Phenolphthalein in Chocolate Preparations (*156*)—Official

32.315 REAGENTS

(**a**) *Potassium hydroxide soln.*—$5 \pm 0.1N$.

(**b**) *Iodine soln.*—0.5*N*. Dissolve 12.7 g KI in 10 ml H_2O, add 6.35 g I, and when dissolved add 12 ml of the KOH soln, (**a**). Dil. to 100 ml with H_2O.

(**c**) *Sodium sulfite soln.*—Dissolve 12.6 g anhyd. Na_2SO_3 in H_2O and dil. to 100 ml with H_2O.

32.316 PREPARATION OF ALCOHOLIC EXTRACT

Chill sample until hard; then reduce to granules by grating, shaving, or grinding. Mix thoroly. Weigh accurately into gooch, with thin asbestos mat or fritted glass disk, quantity of prepd sample contg ca 0.1 g phthln. Ext. fat with 5, 4, and 3 ml CCl_4, using slight suction towards end. Place crucible on bell jar arrangement. Ext. phthln from sample with several portions of hot alcohol, collecting filtrate in 300 ml tall beaker. Wash underside of crucible free from phthln with hot alcohol (ca 50 ml is enough for extn and washings). Evap. combined alc. exts to dryness on steam bath.

32.317 DETERMINATION

Dissolve residue at room temp. in 1–1.5 ml of the KOH soln. (Alk. phthln soln is unstable in

air, and phthln should be converted to tetraiodo compound within 1 hr.) Add piece of ice (ca 40 g) and 7–8 ml of the I soln. Add HCl dropwise from buret, using stirring rod, to complete pptn. If ppt and supernatant are not brown, add more of the I soln to insure excess. Again dissolve ppt by adding the KOH from buret dropwise, with stirring. Wash down any unreacted phthln adhering to sides of beaker with little H_2O. (Soln should now be blue to blue-purple.)

Repeat pptn with acid and resoln with alkali 3 more times, adding small piece of ice if necessary. Then add 1–1.5 ml of the Na_2SO_3 soln to blue alk. soln and filter into 250 ml beaker thru gooch with *thin* asbestos mat or *coarse* fritted glass disk. Wash crucible several times with H_2O. Acidify filtrate with HCl, using few ml excess, and heat on steam bath 20–30 min., stirring occasionally. Decant hot supernatant thru weighed gooch (with asbestos mat or medium fritted glass disk). Wash white to cream-colored ppt in beaker by decantation with hot H_2O few times. Transfer ppt completely to the gooch and wash with hot H_2O until filtrate is clear and gives negative test for Cl. When app. has cooled and ppt has been sucked fairly dry, wash ppt several times with petr. ether, using suction toward end. Dry the tetraiodophenolphthalein to constant wt at 110–130°. Wt ppt $\times$0.3872 = wt phthln.

32.318 Phenolphthalein in Emulsions (*157*)—Official

Shake sample well, preferably in mechanical shaker, 10 min. Weigh accurately quantity of sample contg ca 0.1 g phthln from weighing buret directly into centrifuge bottle. Add 100 ml alcohol-ether (1+3), stopper bottle, shake vigorously, and then centrifuge until clear. Decant into separator. Wash residue in bottle twice with 10 ml portions of the solvent mixt., adding these washings to separator. Dissolve residue in bottle in few ml H_2O and reppt gums with 50 ml of the solvent mixt. Again shake and centrifuge as before, decanting into separator. Wash residue and bottle with three 10 ml portions of the solvent mixt. and add these to separator. Dissolve residue in few ml H_2O and test for complete extn with NaOH.

Shake exts in separator repeatedly with 25 ml portions ca 0.1N NaOH until phthln is completely removed, as shown by absence of color. Combine alk. exts in another separator and acidify soln with dil. H_2SO_4.

Ext. phthln by shaking acid mixt. repeatedly with 10 ml portions ether. Test for complete extn with NaOH soln. Combine ether exts in 150 ml beaker, evap. to dryness, and det. phthln as in **32.317**, omitting filtration of alk. soln.

Phenolphthalein in Tablets

32.319 *Ether Extraction Method (158)—Official*

(Not applicable in presence of other ether extractives)

Weigh portion of powd. sample, **32.002**, contg ca 0.2 g phthln, transfer to separator, using 10 ml 5% NaOH soln and little H_2O, and ext. 3 or 4 times with ether as in **32.001**, using 25 ml for first and 20 ml for each subsequent extn. Transfer ether exts to second separator and wash with two 5 ml portions of the NaOH soln. (Substances like quinine, acetanilid, and acetophenetidin as well as any unsaponified fatty material or mineral oil, if present, are removed by extn with ether.) Combine alk. solns and acidify wtih HCl. Ext. 4 or 5 times with ether as before, until all phthln has been removed, as detd by testing portion of last ether ext. with NaOH soln. Filter ether exts into weighed beaker, evap., dry residue at 100°, and weigh. Residue should be sol. in alcohol, showing absence of most oils. If titrd with 0.1N NaOH, alc. soln should be practically neutral, showing absence of acid extractives such as fatty acids and salicylic or benzoic acid.

Phenothiazine (*159*)—Official

32.320 REAGENT

Phenothiazine std.—Dissolve phenothiazine in 10 parts toluene with heat. Add 0.1 g activated charcoal for each 4 g phenothiazine. Boil 10 min. under reflux and filter while hot thru heated filter. Cool soln, and collect phenothiazine crystals on büchner or fritted glass filter. Dry crystals at 100° and then in vac. desiccator contg paraffin chips. Repeat recrystn process, if necessary, until product melts at 184–185°.

32.321 APPARATUS

Photoelectric colorimeter.—Any photoelec. colorimeter with 510–530 mμ filter or spectrophotometer set at ca 508 mμ is satisfactory. Visual colorimeter may be substituted but accuracy is decreased.

32.322 DETERMINATION

Prep. std curve as follows: Weigh quantities (90–110 mg) of std phenothiazine, develop color as below, obtain readings, and plot mg phenothiazine against readings.

Weigh accurately quantity of sample contg 100 mg ($\pm$10%) phenothiazine and transfer to 300–500 ml g-s. bottle. Add from pipet 200 ml alcohol, stopper bottle, and shake until phenothiazine completely dissolves. Pipet 5 ml clear supernatant into 100 ml g-s. vol. flask. Add 45 ml alcohol and heat 10 min. in H_2O bath at 60°. Add rapidly from graduated cylinder 5 ml satd Br-

H_2O, stopper tightly, and heat 15 min. in H_2O bath at 60°. Add addnl 5 ml satd Br-H_2O, stopper tightly, and let stand 10 min. outside bath. To remove excess Br return open flasks to H_2O bath, heat bath to ca 90°, and continue heating 5 min. after alcohol vapors begin to escape from flask. Cool to room temp. and dil. to mark with alcohol. Det. absorbance (or transmittance) and det. mg phenothiazine from std curve.

Phenylethylamines (*160*)—First Action

(Applicable to amphetamine, methamphetamine, mephentermine, vonedrine, and ephedrine)

32.323 APPARATUS

Spectrophotometer.—Capable of isolating bands of 1 mμ or less in region 250–270 mμ; equipped with 1 cm cells of quartz or fused Si (preferably matched pair).

32.324 PREPARATION OF STANDARD SOLUTION

Accurately weigh 500–700 mg of the phenylethylamine salt of known purity, transfer to 100 ml vol. flask, and dissolve in 0.1*N* H_2SO_4. Dil. to mark with the H_2SO_4 and mix well.

32.325 DETERMINATION

Weigh 20 or more units of sample (tablets or capsules) and obtain av. wt per unit. Grind 5–20 units to uniform 60–80 mesh powder.

Accurately weigh powd. sample contg 25–50 mg amine base and transfer to 40–50 ml g-s. centrifuge tube. Add 5 ml 1*N* H_2SO_4, and swirl tube gently to aid escape of liberated CO_2, if present. Test for acidity with litmus paper, adding more acid if necessary. Pipet in 20 ml $CHCl_3$ and 7 ml 1*N* NaOH, stopper securely, and shake 3–5 min. To second 40–50 ml centrifuge tube add 10 ml of the std soln and ca 0.5 g powd. Na_2SO_4. Swirl tube gently to dissolve salt, pipet in 20 ml $CHCl_3$ and 2 ml 1*N* NaOH, stopper securely, and shake 3–5 min.

Centrifuge tubes at 1500–1800 rpm 3–5 min. Withdraw 10 ml clear $CHCl_3$ layer by closing upper end of 10 ml pipet with index finger while lowering tip thru upper aq. layer. Wipe off any drops adhering to outer portion of pipet, and transfer 10 ml of the $CHCl_3$ layer to separator contg 10–15 ml 0.1*N* H_2SO_4. Stopper separator and shake thoroly, but not violently, 2–3 min. Let $CHCl_3$ layer sep. cleanly; then drain into second separator contg ca 10 ml 0.1*N* H_2SO_4. Shake, let sep., and discard $CHCl_3$ layer which may contain tablet lubricants and neutral products as flavoring materials, dyes, etc.

Wash the two acid solns with 5 ml portion of fresh $CHCl_3$. Discard $CHCl_3$ wash. Filter the two acid solns thru small wad of cotton wet with H_2O in neck of small funnel, collecting filtrate in 50 ml vol. flask. Rinse separators with several small portions of 0.1*N* H_2SO_4, passing rinse solns thru cotton filter into vol. flask. Dil. to mark with 0.1*N* H_2SO_4, stopper, and mix well.

Prep. *blank* acid soln by shaking 25 ml 0.1*N* H_2SO_4 with 3–5 ml $CHCl_3$. Let sep., and drain and discard $CHCl_3$. Filter acid soln thru cotton as above and dil. to 50 ml with 0.1*N* H_2SO_4.

Read absorbance of filtered solns in 1 cm cells at 0.5 mμ intervals in range of 252–255 mμ for first minima, 256–258 mμ for maxima, and 260–262 mμ for second minima. (It is essential to use same slit width for sample and std solns. Best results are obtained with slit widths of 0.4–0.7 mm when using Beckman DU.)

32.326 CALCULATIONS

Calc. absorbance difference (ΔA) between absorbance at maxima and av. of 2 minima: $\Delta A = A_{max} - 0.5(A_{min\ 1} + A_{min\ 2})$.

Calc. absorptivity differential (Δa) produced by 1 g/L (1 mg/ml) of the std amine base or salt: $\Delta a_{std} = \Delta A_{std} \times 100/\text{wt std}$, where ΔA_{std} is absorbance difference for std soln; 100 is ml std soln measured; and wt std is mg std in aliquot measured.

Mg amine/unit of sample

$$= \frac{(\Delta A_{sample} \times 50 \times 2 \times \text{av. wt of unit in mg})}{(\Delta a_{std} \times \text{wt sample in mg})},$$

where 50 is vol. sample soln and 2 is aliquot factor.

Phenylpropanolamine Hydrochloride—First Action

Chromatographic Method (*161*)

32.327 APPARATUS

See **32.133**.

32.328 REAGENT

Chloroform.—Absorbance at 258.5 mμ, measured against H_2O blank, <0.200.

32.329 PREPARATION OF TUBE WITH WASH LAYER

Fix pledget of glass wool in stem of chromatographic tube above constriction. Clamp tube vertically. In small beaker mix 3 g Celite No. 545 and 2 ml H_2O. Transfer to tube with metal spatula and press down evenly with packing rod.

32.330 DETERMINATION

(a) *Capsules and tablets.*—To 150 ml beaker transfer accurately weighed quantity powd. sample contg ca 50 mg phenylpropanolamine.HCl. Add 5 ml NH_4OH (1+4) and mix by gentle swirling. Add 5 g Celite and mix with metal spatula.

Transfer to tube without loss thru powder funnel, in 4 or more portions, pressing down each portion evenly with packing rod. When removing funnel from tube each time, tamp it lightly in tube to remove loosely adhering particles; then hang it in beaker of such size that it does not touch bottom. After using packing rod, scrape off most of adhering material into tube with spatula, and tap rod and spatula over mouth of tube. When laying down implements, place them in such position that their ends do not touch anything. Finally use smooth, intact rubber policeman to sweep material from beaker and funnel into tube. Rub beaker, spatula, and packing rod with three ca 1 g portions Celite, sweeping each portion thru funnel into tube, using rubber policeman for these operations. Press down each portion with packing rod.

Place 100 ml vol. flask in receiving position. Wash down inside of tube with $CHCl_3$, adding enough (ca 20 ml) to moisten column and produce only few drops of eluate. Elute with 95 ml $CHCl_3$, wash tip of tube with little $CHCl_3$, and dil. to vol. with $CHCl_3$. Measure absorbance at 258.5 mμ, 2–5 min. after pouring into silica cell, against portion of same $CHCl_3$ used for elution.

To 150 ml beaker transfer ca 50 mg pure phenylpropanolamine hydrochloride, accurately weighed. Proceed as with sample, beginning "Add 5 ml NH_4OH (1+4) . . ." Det. absorbances of sample and std eluates at ca same time, on same setting of wavelength dial. Use same cell for both eluates, and same cell for both blanks. Calc. phenylpropanolamine.HCl content.

(**b**) *Aqueous solns.*—Prep. tube with wash layer as in **32.329.** Into 150 ml beaker pipet vol. sample contg ca 50 mg phenylpropanolamine .HCl, or pipet 10 ml, whichever is less. Add 1 ml NH_4OH and mix by gentle swirling. Add number of g Celite equal to total number ml of liquid and mix with metal spatula. Proceed as in (**a**), beginning "Transfer to tube without loss . . ."

Extraction Method (162)

32.331 DETERMINATION

Weigh 20 or more units of sample (tablets or capsules) and obtain av. wt/unit. Grind to uniform 60–80 mesh powder. Accurately weigh powd. sample contg 25–50 mg amine base and transfer to 40–50 ml g-s. centrifuge tube contg 3–3.5 g NaCl and 6–7 glass beads. Dissolve sample by adding 5 ml 1*N* H_2SO_4, and swirl tube gently to aid escape of any liberated CO_2. Test for acidity with litmus paper, adding more acid if necessary. Pipet in 25 ml $CHCl_3$ and 7 ml 1*N* NaOH, stopper securely, and shake 3–5 min. To second 40–50 ml centrifuge tube add 10 ml of the std soln, **32.324**, and 3–3.5 g NaCl. Swirl tube gently to dissolve salt, pipet in 25 ml $CHCl_3$ and 2 ml 1*N* NaOH, stopper securely, and shake 3–5 min.

Centrifuge tubes at 1500–1800 rpm 3–5 min. Withdraw 10 ml of the clear $CHCl_3$ layer by closing upper end of 10 ml pipet with index finger while lowering tip thru upper aq. layer. Wipe off any drops adhering to outer portion of pipet, and transfer 10 ml $CHCl_3$ layer to second 40–50 ml centrifuge tube contg 25 ml 0.1*N* H_2SO_4 and 6–7 glass beads. Stopper securely, shake, and centrifuge as above.

Prep. acid blank soln by shaking 25 ml 0.1*N* H_2SO_4 with 3–5 ml $CHCl_3$ and centrifuge to obtain a clear acid soln. Read absorbance of portion of the clear acid soln obtained from aliquot of std and of sample soln against acid blank prepd above in reference cell, using 1 cm cells (preferably matched), at 0.5 mμ intervals as in **32.325.**

32.332 CALCULATIONS

Calc. absorbance difference and absorptivity differential as for phenylethylamines, **32.326.**

Mg amine/unit of sample

$$= \frac{(\Delta A_{\text{sample}} \times 25 \times 25 \times \text{av. wt of unit in mg})}{(A_{\text{std}} \times 10 \times \text{wt sample in mg})}.$$

Propylene Glycol (*163*)—Official

32.333 REAGENTS

Use reagents in **19.005** except to substitute following for heptane:

(**a**) *Cyclohexane.*—Eastman practical grade, b.p. 81° or equiv.

32.334 DETERMINATION

Isolate propylene glycol as in **19.006,** using cyclohexane instead of heptane. Det. propylene glycol as in **19.007**(**a**). To correct for glycerol if present, transfer 25 ml aliquot of the oxidized mixt. to erlenmeyer, add 1 drop bromocresol purple indicator, and titr. with 0.02*N* NaOH to light purple end point. Apply appropriate correction for any acidity in the 0.02*M* KIO_4 soln by titrg 25 ml of the KIO_4 soln by same method. 1 ml 0.02*N* NaOH = 1.84 mg glycerol. Calc. as follows:

$A = 2$(ml $KAsO_2$ for blank − ml $KAsO_2$ for 50 ml oxidation aliquot);

$B = 4$[ml NaOH for 25 ml oxidation aliquot − 2(ml NaOH for blank correction)];

Mg propylene glycol in sample aliquot $= (A - 4B) \times 0.76$.

32.335 **Propylthiouracil** (*164*)—**Official**

Start and complete detn on same day.

Transfer accurately weighed sample contg ca 150 mg propylthiouracil to 200 ml vol. flask, and transfer 150.0 mg pure propylthiouracil to another 200 ml vol. flask as std. To each flask add 150 ml NH_4OH (1+13), washing down necks. Shake

flasks moderately and continuously 1 min. to dissolve propylthiouracil. Dil. to marks with NH_4OH (1+13) and mix.

Filter sample soln, discarding first 25 ml filtrate. Dil. 20 ml aliquot clear filtrate to 200 ml with H_2O in vol. flask (or 25 ml aliquot to 250 ml) and mix. Dil. 20 ml aliquot of this soln to 200 ml in vol. flask (or 25 ml aliquot to 250 ml) and mix. Prep. same double diln of std soln to obtain final concn of 0.0075 mg/ml.

Det. absorbance of final solns of std and sample against H_2O blank in silica cells in spectrophotometer at 234 mμ. Apply cell corrections unless same cell is used for both std and sample. Calc. propylthiouracil content of sample.

Pyrilamine in Cough Sirup (*154*)—First Action

32.336 REAGENT

Pyrilamine std soln.—Transfer 150 mg pyrilamine maleate to 500 ml vol. flask, dissolve in ca 0.1N H_2SO_4, and dil. to vol. with ca 0.1N H_2SO_4. Transfer 5 ml aliquot to 100 ml vol. flask and dil. to vol. with ca 0.1N H_2SO_4. (1 ml = 0.015 mg pyrilamine maleate.)

32.337 DETERMINATION OF PYRILAMINE

Proceed as for detn of methapyrilene, **32.308**, but measure absorbances at 314 mμ. Mg pyrilamine maleate/100 ml sample $= (A \times 100 \times 1.5 \times 10)/(A' \times \text{vol. aliquot})$, where A = absorbance of sample and A' = absorbance of std.

Pyridium (Mallophene) (*165*)—First Action

32.338 REAGENTS

(a) *Titanium trichloride std soln.*—Prep. as in **42.040** and stdze as in **42.041.**

(b) *Light green SF yellowish soln.*—Dissolve 1 g FD&C Green No. 2 in H_2O and dil. to 1 L.

32.339 PREPARATION OF SOLUTION

(a) *Solutions.*—To vol. contg ca 0.1 g pyridium add 10 ml 0.1N HCl and dil. to 100 ml.

(b) *Tablets and jelly.*—Weigh sample (powd. in case of tablets) contg ca 0.1 g pyridium, add 10 ml 0.1N HCl, and dil. to 100 ml.

(c) *Ointments.*—Weigh, in 100 ml beaker, sample contg ca 0.1 g pyridium, stir with ether until ointment base dissolves, and wash into separator with ether and H_2O. Shake thoroly, and drain aq. layer into second separator contg 25 ml ether. Shake, and drain aq. layer into third separator contg 25 ml ether. Shake, and transfer aq. layer to 250 ml beaker. Wash ether layers with alternate 10 ml portions HCl (1+1) and H_2O until no more color is removed, passing each portion of the HCl or H_2O successively thru the 3 separators and finally into beaker. Nearly neutralize combined acid exts with NH_4OH, cool, wash into separator, make ammoniacal, and ext. with 25 ml portions $CHCl_3$ until no more color is removed, filtering $CHCl_3$ thru cotton pledget in stem of separator. Evap. combined $CHCl_3$ exts just to dryness, take up in 10 ml 0.1N HCl, and dil. to 100 ml.

32.340 DETERMINATION

Heat soln to boiling, add 15 g *Na acid tartrate*, and boil 2 min. Add 10 ml of the light green SF yellowish soln and titr. hot with the std $TiCl_3$ soln in current of CO_2. End point is change from green to pale yellow. Perform blank titrn with 10 ml 0.1N HCl, 90 ml H_2O, 15 g Na acid tartrate, and 10 ml of the light green SF yellowish soln, and subtract from vol. $TiCl_3$ previously found. 1 ml 0.1N $TiCl_3$ = 0.00624 g pyridium, $C_{11}H_{11}N_5.HCl$.

Tetrachloroethylene in Mixtures (*166*)—Official

32.341 REAGENT

Metallic sodium.—Place 10 ml xylene and 2 g Na in small g-s. erlenmeyer, adding more xylene if necessary to cover metal. Heat on hot plate until Na is melted. Shake to remove excess vapor, stopper, wrap in towel, and shake vigorously until Na is finely divided. Cool, remove xylene, and replace with 5 ml fresh xylene.

32.342 DETERMINATION

Weigh carefully 125 ml cork-stoppered erlenmeyer. Remove from balance pan, open, and from graduated pipet add enough sample to contain ca 0.16 g tetrachloroethylene. Stopper securely and weigh again. To contents of flask add 10 ml xylene and 2 g of the Na reagent. Connect flask to reflux condenser, using cork stopper protected by Sn foil, and heat to boiling on hot plate. Add ca 1 ml *amyl alcohol* thru condenser. Reflux gently 2 hr and add at intervals 1 ml portions amyl alcohol until total of 5 ml is added. Disconnect flask.

When cool, destroy excess Na by cautious addn of 20 ml H_2O. After all action subsides, acidify with HNO_3 and transfer mixt. to separator. Wash xylene layer with three 10 ml portions H_2O and filter acid aq. solns into 200 ml vol. flask. Add 50 ml 0.1N $AgNO_3$ to flask and dil. to 200 ml. Shake thoroly, and pour thru dry filter, discarding first 20 ml filtrate. To 100 ml aliquot add 3 ml $FeNH_4(SO_4)_2$ indicator, **32.298(c)**. Titr. excess $AgNO_3$, using 0.05N NH_4CNS. Make blank test for Cl. 1 ml 0.1N $AgNO_3$ = 0.00415 g C_2Cl_4. Cl may also be detd gravimetrically as in **32.346**; 1 g AgCl = 0.2892 g C_2Cl_4.

32.343 Thiouracil (*167*)—First Action

Weigh quantity of sample contg 0.25 ± 0.040 g thiouracil and transfer to 100 ml vol. flask. Add ca 50 ml H_2O and then 3 ml 5% NaOH soln, and

shake few min. Dil to vol. with H_2O, shake filter thru dry filter, and transfer 50 ml filtrate to I flask. Add 25 ml 0.5N KBr-$KBrO_3$, **42.018,** and 10 ml HCl (1+3), and let stand exactly 15 min. Then add 10 ml 15% KI soln and let stand exactly 5 min. Titr. the I with 0.1N $Na_2S_2O_3$, taking first disappearance of yellow (or blue) as end point. (Use of starch indicator in titrn is optional.) 1 ml 0.5N KBr-$KBrO_3$ = 0.00641 g thiouracil, $C_4H_4ON_2S$.

Trichloroethylene (*168*)—First Action

32.344 APPARATUS

(a) *Pressure tubes.*—Start with clean, dry piece of soft glass tubing 16.5 cm long, 6–8 mm i.d., with wall 1–1.5 mm thick (1 mm is easier to work). Heat in center and draw out to make i.d. 3–4 mm at narrow point. Seal both ends securely by heating. When cool, cut at narrow point to make 2 tubes, 8–10 mm long. Ends may be sealed after dividing. Leave narrow ends open.

(b) *Covered oil bath.*—Any oil bath is satisfactory if it permits heating of pressure tubes in ca upright position and protects analyst from burn or injury in case tube should burst. Chief risk to guard against is hot oil thrown out of bath. Following app. is suggested:

Wrap large Pyrex test tube, 38×300 mm, with sheet asbestos and wire, preferably so that tube may be slid in and out of wrapping. Leave round bottom exposed. Put heavy mineral oil in tube to depth of ca 10 cm, or enough to cover closed pressure tubes. Support bath in vertical position in hood, with round bottom set into circular hole in piece of asbestos board.

Suspend suitable thermometer from clamp above, with bulb immersed near tubes. Small cylindrical wire basket with attached wire for lowering and raising, fitting into bath tube, may be used to place tubes in bath. Basket and tubes may then be suspended in upper part of bath tube for cooling after reaction. Without basket, simply slip tubes into oil bath loose, and when cool, retrieve by looped wire or other device. In such bath several tubes may be heated at once. Smaller test tube may be used for bath if only one pressure tube is to be heated at time.

32.345 REAGENT

Monoethanolamine.—Colorless and Cl-free. Commercial monoethanolamine purified by distn is usually satisfactory.

32.346 DETERMINATION

Tare pressure tube with suitable support (such as small beaker or wire holder). Using fine-tip pipet, place 0.15–0.17 g sample in tube, gently wiping away any sample on rim or outside of tube. Wait until any sample in upper part of tube has evapd, and weigh. Immediately add 1.0–1.1 ml of the monoethanolamine and immediately seal open end of tube securely in flame, without heating liquids in bottom. When tube has cooled, mix liquids completely and place in the covered oil bath at room temp. (Tube, or tubes, should rest in ca upright position and remain so until opened later.)

Suspend thermometer in bath with bulb near tubes, and heat bath to 210–240°, lowering hood window part way and observing temp. thru window. Keep in this temp. range 1 hr. Discontinue heating, and remove thermometer with tongs to avoid placing hands above bath. Remove tubes from oil, but keep safely covered until cool. (Use of wire basket as described above is convenient, but tubes may instead be allowed to cool in the oil (slower procedure) and then removed. Precautions are not needed after tubes have cooled.)

Remove oil from outside of each tube. Open tube by filing above liquid and breaking cleanly. With aid of H_2O wash bottle, transfer contents without loss to 250–400 ml beaker, and dil. to 100–120 ml with H_2O. If necessary to remove glass particles, filter thru small cotton pledget into second beaker, washing thru entire soln. Neutralize with HNO_3 (10–15 drops) and add 1–1.5 ml excess. Heat to 65–70° and add excess of $AgNO_3$ soln, 5% or less concd. Coagulate on steam bath with occasional stirring, filter thru gooch or fritted glass crucible, wash with hot H_2O, and dry at 130–140° (30 min. is usually enough). 1 g AgCl = 0.30557 g C_2HCl_3.

VEGETABLE DRUGS AND THEIR DERIVATIVES NOT CONTAINING ALKALOIDS

32.347 **Aloin (*169*)—Official**

(Applicable to mixts contg cascara, rhubarb, senna, and other acid hydrolyzable anthraglucosides, as well as to resins and phthln with aloin. Not applicable to aloes.)

Dry enough powd. material 1 hr at 110° (or dealcoholized soln if liquid) to provide ca 0.3 g aloin. Add 10 ml H_2O and few ml 5% NaOH soln. Transfer mixt. to 100 ml vol. flask, dil. to ca 75 ml, and immediately acidify with H_2SO_4, as aloin is attacked by alkali. Dil. to mark, and add few glass beads if much undissolved material is present. Shake occasionally during 1 hr to insure soln of aloin. Filter, transfer 40 ml aliquot to continuous extn app. previously charged with $CHCl_3$ (*A*, Fig. 63, page 475) and add 10 ml 10% H_2SO_4 (by wt) to the aliquot. Reflux to exhaustion (ca 2 hr).

Disconnect app. and transfer all aq. soln to separator, discarding $CHCl_3$. Sat. soln with NaCl and shake out with five 30 ml portions $CHCl_3$-alcohol mixt. (3+1). Test for complete removal of aloin by evapg portion of sixth extn (more exts may be necessary). Shake violently. Combine exts, add 1 ml H_2O and 1 g $NaHCO_3$, or more if necessary to insure excess, and shake. Filter sol-

vent, evap., add 5 ml $CHCl_3$, evap., dry 1 hr at 110°, cool, and weigh rapidly. Wt = aloin in aliquot taken.

As check, acetylate the aloin by dissolving in ca 10 ml Ac_2O, adding excess (ca 2 g) of powd. anhyd. NaOAc, and boiling 5 min. in acetylation flask placed in oil bath. Wash sample from flask with addnl Ac_2O and evap. to apparent dryness in hood with good draft. Add 10 ml H_2O and heat several min. Transfer with $CHCl_3$ to separator, washing flask with successive portions of $CHCl_3$, and shake out with 2 addnl 10 ml portions $CHCl_3$ (aloin hexaäcetate formed is sol. in $CHCl_3$). Combine $CHCl_3$ portions, filter, evap., add 10 ml $CHCl_3$, evap., dry 1 hr at 110°, cool, and weigh. Wt × 0.615 = aloin.

32.348 Camphor (*170*)—**Official**

(Not applicable to synthetic camphor)

Weigh accurately, into 400 ml round-bottom Pyrex flask, enough powd. sample to contain ca 2 g camphor. Add 10 ml benzene and 10 ml H_2O, and connect flask with app. for steam distn. Use 8–12″ bulb condenser, well-cooled, outlet of which reaches to bottom of 200 ml flask. Steam distill, collecting the benzene and ca 100 ml aq. distillate. Disconnect condenser and wash it slowly with 5 ml alcohol from pipet so as to wet entire inside of condenser. Wash condenser similarly with 10 ml benzene. Add both washings to contents of receiver.

Sat. distillate with NaCl, add enough H_2SO_4 (1+9) to insure acidity, transfer to separator, shake, and sep. 2 layers. Rinse original receiver with 10 ml benzene and use rinsing to re-ext. aq. soln. Sep. aq. layer and ext. it once more with 10 ml benzene. Wash combined benzene exts with 10 ml satd NaCl soln rendered distinctly alk. with Na_2CO_3. Sep. layers and ext. aq. layer with 10 ml benzene. Discard aq. solns, transfer benzene to 50 ml vol. flask, and dil. to mark with benzene. Shake soln and filter into 200 mm polariscope tube, using H_2O-jacketed tube, if necessary, to keep constant temp. of 20°. Make 10 readings, using $K_2Cr_2O_7$ filter, **29.020(b)**, and take av. reading for calcg the camphor. Calc. quantity of camphor, Q, contained in the 50 ml benzene and, therefore, in sample taken, from av. reading in circular degrees, a, by formula: $Q = 0.6171a - 0.0022a^2$.

Value of Q does not vary directly with length of tube. If longer or shorter tube than directed is used, correct value of a to 200 mm tube, and then make calcn by above formula.

Camphor in Spirits (*171*)—**Official**

32.349 REAGENT

2,4-Dinitrophenylhydrazine reagent.—Dissolve 2 g 2,4-dinitrophenylhydrazine in 20 ml cold H_2SO_4 (1+1) by shaking in g-s. flask; add 35 ml H_2O, mix, cool, and filter.

32.350 DETERMINATION

Dil. accurately measured quantity of spirit of camphor with *aldehyde-free alcohol* until soln contains ca 0.2 g camphor/10 ml. Pipet 10 ml diln into 125 ml pressure bottle contg 50 ml of the freshly prepd dinitrophenylhydrazine reagent. Close pressure bottle, immerse in beaker of H_2O, and heat on steam bath 4 hr, keeping temp. of pressure bottle at ca 75°. Cool to room temp.; then transfer contents to beaker, using 100 ml H_2SO_4 (1+11). Let stand overnight at room temp. Collect ppt on tared gooch; wash with 10 ml of the dil. H_2SO_4, and then with 75 ml cold H_2O to remove acid. Dry at 100°. Wt ppt × 0.458 = wt camphor.

Camphor, Monobromated, in Tablets

(*172*)—*Official*

32.351 REAGENT

Sodium amalgam.—Cut ca 1 g bright metallic Na into small pieces and dissolve in 100 g warm Hg, contained in small porcelain mortar, by impaling pieces successively on point of file and holding them submerged in the Hg until rather violent action is complete. Keep resulting amalgam in tightly corked bottle.

32.352 DETERMINATION

Weigh portion of powd. sample contg 0.1–0.2 g monobromated camphor and transfer with 20 ml alcohol and 10 ml H_2O to 100 ml round-bottom flask contg 15 g of the Na amalgam. Connect flask by means of rubber stopper with vertical condenser. Boil mixt. gently over wire gauze at least 30 min. Cool slightly and wash out condenser tube with 5 ml alcohol and 5 ml H_2O, receiving washings in flask.

Place flask on steam bath and heat another hr, or until evolution of H has nearly or quite ceased. Toward latter part of this operation, to facilitate reduction, make liquid ca neutral with few drops of HOAc. Transfer contents of flask to separator, withdrawing Hg into second separator and washing it with at least two 50 ml portions H_2O. Pass the several aq. solns thru small filter, collecting clear filtrate in suitable beaker. Ppt with 10% $AgNO_3$ soln, add ca 5 ml HNO_3, and filter, collecting AgBr in weighed gooch. Wash with H_2O and alcohol, dry at 100°, and weigh. Wt AgBr × 1.23 = wt monobromated camphor. Perform control detn on amalgam to det. if correction is necessary.

Chenopodium Oil (*173*)—**Official**

32.353 REAGENTS

(a) *Ferric ammonium sulfate std soln.*—0.1N. Dissolve 39.214 g pure, crystd $Fe(NH_4)_2(SO_4)_2$

$.6H_2O$ in 200 ml H_2O in 1 L flask, add 30 ml H_2SO_4, and mix well. Weigh exactly 3.16 g $KMnO_4$, dissolve in 200 ml warm H_2O, and slowly add to soln in flask, with stirring. ($KMnO_4$ soln should be just enough to oxidize ferrous salt, but add last few ml in small portions.) Cool soln and dil. to 1 L with H_2O.

(b) *Titanium trichloride std soln.*—Add 100 ml commercial 15–20% $TiCl_3$ soln to 200 ml HCl, boil 1 min., cool, and dil. to 4.5 L with H_2O. Place soln in container with H atmosphere provision and let stand 2 days for absorption of residual O. Preserve $TiCl_3$ soln under H, Fig. 68 p. 587, taking care to have all joints air-tight, and covering stoppers (preferably countersunk) with suitable wax. Stdze by titrg 20 ml of the $FeNH_4(SO_4)_2$ soln against the $TiCl_3$ soln in protective stream of CO_2, using 1 ml 5% NH_4CNS soln as indicator. 1 ml 0.1*N* $FeNH_4(SO_4)_2$ = 0.01545 g $TiCl_3$.

32.354 DETERMINATION

Weigh 1 ml sample in 100 ml vol. flask and dil. to vol. with alcohol.

Place 50 ml of the $TiCl_3$ soln in erlenmeyer thru which passes current of CO_2. Fit flask with Bunsen valve, add 10 ml dild soln of the oil, close flask with the Bunsen valve, and heat contents almost to boiling 2 min. (Prolonged heating has no effect if contents are not boiled vigorously.) If pale violet color of the $TiCl_3$ disappears, add more reagent to insure excess. (Formation of white ppt does not interfere with detn.) Add 1 ml 5% NH_4CNS soln and titr. excess $TiCl_3$ with the $FeNH_4(SO_4)_2$ soln in CO_2 atmosphere to faint permanent brownish-red.

Subtract quantity of $FeNH_4(SO_4)_2$ soln used, expressed in equiv. mg $TiCl_3$, from mg $TiCl_3$ taken. Difference is mg $TiCl_3$ oxidized by oil taken. To convert mg $TiCl_3$ oxidized into ascaridole, divide by factor 1.284 (1 g ascaridole is reduced by 1.284 g $TiCl_3$).

EXAMPLE: 0.9600 g oil was dild to 100 ml and 10 ml aliquot was heated with 50 ml of the $TiCl_3$ soln (1 ml contg 0.0034 g $TiCl_3$). Back-titrn required 5.9 ml of the reagent, each ml equiv. to 0.01545 g $TiCl_3$. Number of g $TiCl_3$ oxidized is numerically equal to $(50\times0.0034)-(5.9\times0.01545)$, or 0.07885. Wt oil in aliquot was 0.0960 g. Hence % ascaridole $=0.07885\times100/0.096\times1.284=72.1$.

Digitoxin (*174*)—First Action

32.355 REAGENTS

(a) *Formamide.*—Shake 1 L $HCONH_2$ (99% grade) with ca 30 g anhyd. K_2CO_3 15 min. and filter. Distill under vac. in all-glass app. Reject first portion of distillate contg H_2O, and collect fraction boiling at ca 101°/12 mm Hg (115°/25 mm Hg). Store over H_2SO_4 until odor of NH_3 is no longer detected.

(b) *Alkaline picrate reagent.*—Mix 20 ml 1% aq. picric acid soln with 10 ml 5% NaOH soln, dil. to 100 ml with H_2O, and mix. Reagent is stable 2–3 days.

(c) *Digitoxin std solution.*—Dissolve 20.0 mg USP Reference Standard Digitoxin in alcohol, and dil. to 50 ml with alcohol. Dil. 10.0 ml of this stock soln to 100 ml with alcohol.

(d) *Diatomaceous silica support.*—Celite 545.

32.356 PREPARATION OF CHROMATOGRAPHIC COLUMN

Chromatographic tube.—Prep. chromatographic tube from 25×200 mm test tube. Fit it with packing rod, ca 400 mm long, having flat head 22–23 mm diam. Place small wad of cotton in bottom of tube.

Wash layer.—Add ca 2 g Celite to 1 ml H_2O in 100 ml beaker. Mix thoroly with stirring rod or scoop until the mixt. appears fluffy and uniform, and transfer to chromatographic tube. Press down lightly with packing rod. (Wash layer should be 15–20 mm thick.)

Trap layer.—Add 3 g Celite to 3 ml formamide-H_2O soln (2+1) in 150 ml beaker, mix thoroly, and transfer to tube. Press trap layer down lightly and evenly.

32.357 PREPARATION OF SAMPLE

(a) *Crystalline digitoxin.*—Dissolve 20 mg digitoxin, accurately weighed, in 20 ml $CHCl_3$. Transfer to 100 ml vol. flask with several portions of benzene, dil. to 100 ml with benzene, and mix. Transfer 10.0 ml to chromatographic column. When liquid has passed into column, proceed as in 32.358.

(b) *Tablets.*—Mix thoroly powd. sample contg 2 mg digitoxin with 2 ml H_2O in 250 ml beaker. Add 4 ml formamide, stir thoroly, and cover beaker with watch glass. Heat mixt. 20 min. on steam bath, with frequent stirring. Cool, add 2 ml H_2O and ca 8 g Celite. Stir thoroly until mass appears uniform and does not stick to beaker. Transfer mixt. quantitatively to chromatographic tube thru powder funnel in several portions, pressing it down with stirring rod. Use rubber policeman to sweep adhering particles from beaker and funnel into tube. Scrub beaker and stirring rod with ca 1 g Celite, and add dry washings to tube thru funnel. Repeat washing with 2 addnl portions Celite. Place cotton wad in tube and press it down on column with packing rod, sweeping Celite on sides of tube before it. (Over-all height of column should be 120–150 mm.)

32.358 SEPARATION OF DIGITOXIN

Elute digitoxin with ca 240 ml benzene-$CHCl_3$ (3+1), collecting eluate in 250 ml vol. flask at

rate not >4 ml/min. Wash stem with stream of $CHCl_3$, dil. to 250 ml with $CHCl_3$, and mix.

Continue elution for **32.360.**

32.359 COLORIMETRIC DETERMINATION

Transfer 25 ml aliquot eluate to small erlenmeyer and evap. to dryness on steam bath with aid of air current. Moisten residue with ca 0.5 ml alcohol, and again evap. to dryness. Add 5.0 ml alcohol to cooled flask, stopper, and let stand 15 min. with occasional shaking.

Transfer 5.0 ml aliquot of the std digitoxin soln to small flask and 5 ml alcohol to another flask as blank. Add 3.0 ml of the alk. picrate reagent to blank and to sample and std solns, and mix by swirling. Protect solns from intense light. After 10 min. det. absorbances of std and sample solns relative to blank at 495 mμ, repeating measurements at 2 min. intervals until max. values are attained. Calc. digitoxin content of sample.

32.360 TESTS FOR OTHER DIGITOXOSIDES

After digitoxin seps, elute other digitoxosides with 200 ml $CHCl_3$, collecting eluate in separator. Shake with 100 ml H_2O. Transfer lower layer to beaker, ext. H_2O with 30 ml $CHCl_3$, and add $CHCl_3$ washings to beaker. Evap. to dryness. Pipet 5 ml of the dild digitoxin std soln into second beaker and evap. to dryness. Add 4 ml of the Keller-Kiliani reagent, **32.361(b)**, to each of the cooled residues and mix thoroly. After 15 min., filter thru glass wool if necessary, and det. absorbance of *clear* sample and std relative to reagent blank, at 590 mμ; repeat measurements at 5 min. intervals until max. values are attained. Calc. content of other digitoxosides in sample as digitoxin.

Digoxin and Total Digitoxosides (*175*)—First Action

32.361 REAGENTS

(**a**) *Alkaline dinitrobenzene soln.*—(1) Prep. 5% soln *m*-dinitrobenzene in benzene, and store in g-s. brown glass bottle. (2) Mix 1 ml 10% tetramethylammonium hydroxide soln with 140 ml absolute alcohol, titr. with 0.01*N* HCl, using Me red, and adjust to 0.008*N* with absolute alcohol. Just before use, mix 60 ml (1) with 40 ml (2).

(**b**) *Keller-Kiliani reagent.*—Mix 60 ml HOAc with 1 ml 9% $FeCl_3.6H_2O$ soln and 5 ml H_2SO_4, and cool.

(**c**) *Digoxin std soln.*—25.0 mmg/ml. Dissolve 25.0 mg USP Reference Standard digoxin, $C_{41}H_{64}O_{14}$, in hot alcohol, cool, dil. to 100 ml, and mix. Dil. 10.0 ml of this soln to 100 ml with alcohol and mix.

32.362 PREPARATION OF SAMPLE

(**a**) *Crystalline digoxin.*—Prep. alc. soln contg 125 mmg digoxin/ml. Transfer 10.0 ml to separator, add 50 ml H_2O and 1 ml 2*N* H_2SO_4, and ext. with three 30 ml portions $CHCl_3$. Wash each $CHCl_3$ ext. in second separator by shaking with 10 ml H_2O and 1 g powd. *anion-cation exchange resin* (Rohm and Haas Amberlite MB-1, analytical grade, indicator-free, has been found satisfactory), and filter thru pledget of cotton moistened with $CHCl_3$ into 100 ml vol. flask. Dil. to mark with $CHCl_3$ and mix well. This soln is *Assay Soln.*

(**b**) *Elixirs and injections.*—Transfer aliquot contg 1.25 mg digoxin to separator, and proceed as for crystalline digoxin, (**a**), beginning: "add 50 ml H_2O and 1 ml 2*N* H_2SO_4 . . . "

(**c**) *Tablets.*—Weigh accurately, into 100 ml beaker, portion of powd. tablets contg 1.25 mg digoxin. Add 10 ml alcohol, cover with watch glass, and heat to simmering on steam bath. Let simmer 20 min. with frequent stirring. Cool, wash quantitatively into separator with 30 ml $CHCl_3$ and 50 ml H_2O, add 1 ml 2*N* H_2SO_4 and proceed as for crystalline digoxin, (**a**), beginning: "ext. with three 30 ml portions $CHCl_3$."

32.363 DETERMINATION

(**a**) *Digoxin.*—Pipet 5.0 ml digoxin std soln and 10.0 ml assay soln into similar erlenmeyers, and evap. to dryness on steam bath with aid of air current. Cool, and to each flask add 5.0 ml freshly prepd alk. dinitrobenzene reagent. Let stand 5 min. at temp. not $>30°$, with frequent mixing. Det. absorbances of developing blue colors relative to reagent blank at 620 mμ at 1 min. intervals, using matched 1 cm cells and spectrophotometer. Record max. absorbance of aliquot of assay soln as A and that of digoxin std soln as A'. Digoxin (mg in assay soln) $= 1.25\ A/A'$.

(**b**) *Other digitoxosides.*—Pipet 20.0 ml assay soln and 10.0 ml digoxin std soln into sep. beakers and evap. to dryness on steam bath with aid of air current. Cool, add 4.0 ml Keller-Kiliani reagent at temp. not $>30°$ to each beaker, and mix thoroly. After 15 min., det. absorbances of sample and std at 590 mμ relative to reagent blank at 5 min. intervals. Record max. absorbance of sample as A and that of std as A'. Total digitoxosides calcd as digoxin (mg in sample soln) $= 1.25\ A/A'$. Difference between this value and that obtained in (**a**) is quantity of other digitoxosides in sample soln.

Gums, Identification (*176*)—Official

(*See* also **15.172–15.178**)

32.364 REAGENTS

(**a**) *Iodine-potassium iodide in zinc chloride soln.*—To 100 ml 60% $ZnCl_2$ soln, sp. gr. 1.8, add soln of 10 g KI and 0.15 g I in 10 ml H_2O. Keep few crystals of I in the soln.

(b) *Alcoholic iodine soln.*—Dissolve 7 g I and 5 g KI in 5 ml H_2O and dil. to 100 ml with alcohol.

(c) *Ruthenium red soln.*—To few ml 10% $Pb(OAc)_2.3H_2O$ soln, add enough ruthenium red $[Ru_2(OH)_2Cl_4.7NH_3.3H_2O]$ (available from K&K Labs., Inc., 177-10 93rd Ave., Jamaica 33, N.Y.) to produce wine red color.

(d) *Alcoholic methylene blue soln.*—0.1% soln in alcohol.

(e) *Aqueous methylene blue soln.*—0.1% soln in H_2O.

32.365 PREPARATION OF SAMPLES

(a) *Controls.*—Moisten 1 g of the dry gum with alcohol, add 100 ml H_2O with constant stirring, and bring to boil. To 5 or 10 ml resulting liquid or jelly add 4 vols alcohol, mix, and centrifuge to bring ppt together as compact mass. (Some gums, notably acacia and agar, may fail to be thrown down by this treatment. Addn of few drops of satd NaCl soln should cause rapid flocculation and settling.)

(b) *Jellies or lotions.*—Stir, and add H_2O if necessary to produce fluid mass. Treat portion of sample with alcohol to ppt the gum as in (a). Remove fatty or oily material, if present, by washing pptd gum with ether; then redissolve in H_2O and reppt.

32.366 DETERMINATION

With clean towel squeeze small lump of the alcohol ppt against slide to form mat 4–8 mm diam. on slide. Note character of resulting mat as possible index to type of gum. Quince and Irish moss form thin and rather translucent films, while agar, starch, and acacia are white and opaque. Cover mat with large drop of the I-KI-$ZnCl_2$ soln and observe carefully both with and without magnification. For direct examination place slide upon white surface. For microscopic examination use magnification of ca 90×. If no characteristic color is produced within 1–2 min., proceed with fresh mat to examine for the next group, **32.367**. Continue similarly, using fresh mat for each test thru all group tests until identified.

32.367 *Characteristics of tests for gums*

Group I.—Reagents: Iodine-potassium iodide in zinc chloride soln

GUM	ORIGINAL ALCOHOL PPT	GROUP REACTION	CONFIRMATORY TEST	REMARKS
Tragacanth	Stringy Bluish Translucent	Blue	Warm with 10% NaOH soln on steam bath. Yellow	Certain gums, *e.g.*, Irish moss, may yield dull yellow color with NaOH. Tragacanth bright yellow
Starch	White Compact	Blue-black	Iodine, 0.1*N* Blue	Tragacanth may yield faint blue
Quince	Stringy Translucent	Blue	Above tests negative	Quince is distinguished from starch and tragacanth by negative reactions
Irish moss	Stringy	Brown (small blue particles)	Characteristic nodular structures with group reagent	Old prepns of this gum may fail to show characteristic structures

Group II.—Reagent: Alcoholic iodine soln
(Let soln dry on mat, flush off with alcohol, and irrigate with H_2O)

Agar	White Opaque	Opaque Blue-black	Stains with ruthenium red	Does not dissolve or lose shape when covered with H_2O
Irish moss	Stringy	Brown or lilac	Characteristic blue stain with alc. methylene blue	Reactions yielded by old as well as fresh prepns

Group III.—Reagent: Ruthenium red

Karaya	Fine flocculent compact mass on centrifuging	Swells considerably. Strongly stained pink gran. mass.	Heat with HCl. Pink	Aq. methylene blue produces characteristic blue stain

Group IV.—Reagent: H_2SO_4
(Warm cautiously on steam bath)

Carob bean	Stringy	Pink or red-brown	No satisfactory test found	Alcohol ppt from carob bean gum resembles that from tragacanth
Acacia		Greenish brown	Ppt completely sol. in H_2O	Complete soln of acacia distinguishes it from most other gums

Ipomea (*177*)—Official

32.368 DETERMINATION OF RESIN

Place 10 g sample, as "60-mesh" powder, in 250 ml erlenmeyer and add 50 ml alcohol. Fit flask with stopper thru which is inserted glass tube ca 1 m long to act as condenser, and heat on gently simmering steam bath 30 min., shaking occasionally. Transfer contents of flask to small percolator and percolate slowly with warm alcohol until ca 95 ml tincture collects.

To det. whether extn is complete, collect 10 ml more percolate and pour few drops into cold H_2O; if more than faint cloudiness appears, continue percolation with warm alcohol until test for resin fails. Conc. the addnl percolate by evapn and add to flask before dilg to vol. Cool percolate to room temp. and dil. to 100 ml with alcohol. Mix well.

Evap. 25 ml of the prepd tincture (representing 2.5 g drug) on H_2O bath in beaker or flask of suitable size and dry residue until alcohol-free. Add 15 ml H_2O, bring mixt. to boiling, let cool ca 3 min., and stir well with flat-end rod 2 min. to insure thoro washing of resin. Cool mixt. by placing container in jar of ice-cold H_2O and decant wash H_2O onto 9 cm filter. Repeat washing of resin with another 15 ml portion H_2O, boiling and cooling mixt., kneading resin as before, and decanting washings into filter, as described previously. Repeat washing and kneading process with hot H_2O third time.

Dissolve residue in container in 10 ml warm alcohol and pour soln onto filter, collecting filtrate in weighed beaker or flask. Use enough hot alcohol in small portions to completely transfer soln of resin to filter and insure thoro washing of filter. Evap. combined filtrate and washings to apparent dryness, add 1 ml absolute alcohol, and evap. solvent, taking care to rotate container in inclined position as last portions of solvent are dissipated. Dry residue at 80° to constant wt.

32.369 Jalap (*177*)—Official

Proceed as in 32.368.

32.370 Menthol (*178*)—Official

Weigh 5 g menthol into 100 ml acetylation flask, and add 10 ml Ac_2O and 1 g powd. anhyd. NaOAc. Boil mixt. gently 1 hr, cool, and disconnect flask from condenser, transferring mixt. to small separator. Rinse acetylation flask with three 5 ml portions warm H_2O and add rinsings to separator. After complete sepn, drain aq. layer, and wash remaining oil with successive 5 ml portions Na_2CO_3 soln (12.5 g in 200 ml H_2O) until mixt. is alk. to 2 drops phthln. Dry resulting oil with fused $CaCl_2$ and filter.

Transfer 4–5 ml of the dry acetylated oil to tared 100 ml erlenmeyer, note exact wt, add 50 ml 0.5N alc. KOH, connect flask to reflux condenser, and boil mixt. on H_2O bath 1 hr. Let mixt. cool, disconnect flask from condenser, and titr. excess alkali with 0.5N H_2SO_4, using 10 drops of the phthln as indicator. Calc. % menthol by following formula: % total menthol $= A \times 7.813 / [B-(A \times 0.021)]$, where A is result obtained by subtracting ml 0.5N H_2SO_4 required in titrn from ml 0.5N alc. KOH originally taken, and B is wt acetylated oil taken.

Podophyllum (*179*)—Official

32.371 DETERMINATION OF RESIN

Place 10 g sample, as "60-mesh" powder, in 250 ml erlenmeyer and add 35 ml alcohol. Fit flask with stopper thru which is inserted glass tube ca 1 m long to act as condenser, and heat mixt. on gently simmering steam bath 30 min., shaking occasionally. Transfer contents of flask to small percolator and percolate slowly with hot alcohol until ca 95 ml percolate collects. Collect ca 10 ml more percolate in sep. container. Cool first percolate to room temp. and dil. to 100 ml with portion of second percolate.

Place 50 ml alc. soln in tared beaker and add 2 ml H_2O. Evap. until percolate weighs 3 g. If wt should fall below 3 g, add alcohol dropwise to make to 3 g. Pour residue slowly, with constant stirring, into second beaker contg 10 ml H_2O previously mixed with 1 ml 1N HCl and cooled to <10°. (Pellets of ice placed in beaker and renewed from time to time serve well.) Add 5 ml H_2O and few drops of HCl (1+3) to tared beaker, stir well, and rub sides of container with glass rod. Add mixt. to second beaker and let stand overnight in refrigerator.

Decant supernatant into tared gooch and

transfer ppt to crucible with small portions of cold H_2O slightly acidulated with HCl. (If preferred, collect ppt on filter paper and, after washing, dissolve in hot alcohol, collecting soln in tared beaker.) Dry contents of crucible at 80° and weigh. If particles of resin adhere to either beaker, dissolve them in alcohol, evap. solvent in tared beaker, and dry residue at 80°. Cool, weigh, and add total net wt to wt contents of crucible.

Rutin (*180*)—Official

32.372 REAGENTS

(**a**) *Acid-alcohol reagent.*—Mix 550 ml alcohol with 50 ml HOAc and dil. to 1 L with H_2O.

(**b**) *Rutin std soln.*—0.02 mg/ml. Weigh accurately 100 mg Reference Standard Rutin (obtainable from National Formulary, 2215 Constitution Ave., Washington 7, D. C.) and dissolve in 50 ml of the acid-alcohol reagent. Transfer to 250 ml vol. flask with small portions of the acid-alcohol reagent. Dil. to vol. with the reagent and mix well. Pipet 5 ml aliquot into 100 ml vol. flask and dil. to vol. with H_2O.

(**c**) *Quercetin std soln.*—0.01 mg/ml. Prep. as in (**b**), using 50 mg quercetin. Pure quercetin may be prepd as in reference *181*.

32.373 APPARATUS

(**a**) *Spectrophotometer.*—Capable of isolating following wavelengths: 338.5, 352.5, and 366.5 mμ; with isolated spectrum not wider than 5 mμ.

(**b**) *Absorption cells.*—Matched 1 cm.

(**c**) *Glass stirring rods.*—Of small enough diam. to dislodge material from tips of 50 ml conical centrifuge tubes.

32.374 PREPARATION OF SAMPLE SOLUTION

Weigh directly into 50 ml centrifuge tube number of tablets required to give 0.05–0.50 g rutin (not <5 tablets). Record number and wt. (If tablets are coated, dissolve coating with distd H_2O after weighing, discard aq. washings, and transfer rutin-contg core to centrifuge tube.) Add 20 ml of the acid-alcohol reagent and break up tablets with stirring rod. After tablets are thoroly disintegrated, heat mixt. 10 min. in H_2O bath held at 70–80°, resuspending material occasionally by stirring. Remove stirring rod, rinse with the acid-alcohol reagent, and centrifuge 15 min. at ca 2000 rpm.

Decant supernatant into 250 ml vol. flask, using funnel and decanting with one smooth motion, and let tube drain ca 10 sec. While still inverted, rinse mouth of tube with the acid-alcohol reagent. Ext. twice more, starting with "Add 20 ml of the acid-alcohol reagent . . ." After third extn, dil. combined supernatants to 250 ml with acid-alcohol reagent. Any insol. material may be removed by filtration after diln if first 15–20 ml filtrate is discarded. Depending on original wt rutin taken, make diln with H_2O to give final concn of 0.01–0.03 g rutin/L. Ppts forming during aq. diln may be removed by filtration if first portion of filtrate is discarded to guard against concn changes due to adsorption.

32.375 DETERMINATION

Det. absorbance of sample soln, A_S, against H_2O blank at 338.5, 352.5, and 366.5 mμ. Also det. absorbances of the std rutin soln, A_R, and the std quercetin soln, A_Q, against H_2O blank at 352.5 and 366.5 mμ. (In absence of std quercetin, values $A_{Q,352.5} = 0.553$ and $A_{Q,366.5} = 0.631$ may be used. Any error introduced by use of these predetd values should be of second order.) Calc. as follows:

$$R_1 = A_{S,\ 338.5\ m\mu}/A_{S,\ 352.5\ m\mu};$$

and

$$R_2 = A_{S,\ 366.5\ m\mu}/A_{S,\ 352.5\ m\mu}.$$

If $R_1 = 0.914 \pm .009$ and $R_2 = 0.842 \pm .013$, extd material can be considered pure rutin and wt rutin /tablet can be calcd from following equation:

Mg rutin/tablet

$$= A_{S,\ 352.5\ m\mu} \times d \times W \times 0.02/A_{R,\ 352.5\ m\mu} \times w,$$

where d = sample diln factor; W = av. wt/tablet; w = wt sample.

(Value of R_1 beyond its upper limit while R_2 remains within its range indicates interfering absorption which diminishes rapidly enough to be ineffective at 352.5 mμ. Under this condition, absorbance observed at 352.5 mμ is accepted as correct, and rutin content is calcd as for pure rutin. Increase in R_2 while R_1 remains within or below its limits usually indicates presence of quercetin. Simultaneous increase or decrease of both ratios beyond their respective limits indicates invalidating condition.) Quantities of rutin and quercetin may be calcd by solution of following simultaneous equations:

$$A_{S,\ 352.5\ m\mu} = (A_{R,\ 352.5\ m\mu} \times r/0.02) + (A_{Q,\ 352.5\ m\mu} \times q/0.01)$$

$$A_{S,\ 366.5\ m\mu} = (A_{R,\ 366.5\ m\mu} \times r/0.02) + (A_{Q,\ 366.5\ m\mu} \times q/0.01)$$

where r = mg rutin/ml in sample soln, and q = mg quercetin/ml in sample soln.

Santonin in Mixtures—Official

32.376 *Langer Method* (*Modified*) (*182*)

Weigh sample contg ca 0.15 g santonin, and ext. with 10, 10, 10, 5, and 5 ml portions petr. ether satd with santonin. (If sample is fat-free, this

step may be omitted.) Filter each portion of solvent with aid of suction to complete dryness thru gooch provided with asbestos mat before following with another portion of fresh solvent. Ext. residue in soln flask and crucible with 15, 10, 5, and 5 ml hot benzene, filtering each portion as before. Evap. benzene ext. in tared flask and dry residue to constant wt at 100°. Wt residue in flask = wt santonin in sample.

Dinitrophenylhydrazine Method (183)

32.377 REAGENT

Dinitrophenylhydrazine sulfate soln.—Dissolve 1 g 2,4-dinitrophenylhydrazine in mixt. of 90 ml H_2O and 10 ml H_2SO_4 by warming; cool, and filter.

32.378 DETERMINATION

Weigh 2.5 g ground sample into gooch and wash with ca 100 ml petr. ether satd with santonin. Discard washings. Ext. with ca 100 ml hot benzene, collecting filtrate in beaker. Evap. to dryness, warm residue with alcohol until dissolved, transfer to 100 ml vol. flask, cool, dil. to vol. at 20° with alcohol, and filter if necessary. To 25 ml of this soln add 50 ml of the dinitrophenylhydrazine sulfate soln and let stand 48 hr in dark. Collect ppt in gooch and wash with ca 150 ml alcohol (1+2). Dry residue 1 hr at 100°, cool, and weigh. Wt ppt $\times 0.5775$ = wt santonin.

32.379 Santonin in Santonica (Levant Worm Seed) (*184*)—Official

Ext. 3 g ground sample 3 hr with benzene in Soxhlet app. or automatic percolator, Fig. 64, page 500. Wash ext. into separator with little benzene, add more benzene, if necessary, to make total ca 100 ml, and shake vigorously 5 min. with 35 ml 8% Na_2CO_3 soln. After complete sepn drain aq. layer into second separator. Wash benzene once with 10 ml H_2O and add washing to second separator. Shake combined aq. exts with 10 ml benzene, discard aq. layer, wash benzene with 5 ml H_2O, and combine with benzene in first separator. Filter benzene soln thru cotton and evap. filtrate to dryness.

Warm residue with 5 ml alcohol until mass disintegrates, and add 60 ml satd aq. $Ba(OH)_2$ soln while stirring. Heat mixt. to boiling, place on steam bath 10 min., filter into separator, and wash filter and beaker with two 10 ml portions hot $Ba(OH)_2$ soln. Add 6 ml HCl (2+1) to filtrate, cool, and ext. with 25, 15, 10, 10, and 5 ml portions $CHCl_3$, filtering thru cotton pledget in stem of funnel. Evap. filtrate to dryness, dissolve residue in 25 ml alcohol by warming, mix soln with 50 ml of the dinitrophenylhydrazine sulfate soln, **32.377**, and proceed as in **32.378**, beginning "let stand 48 hr . . ."

32.380 Volatile Acidity of Tragacanth (*185*)—Official

Quantity of volatile (acetic) acidity developed in acid hydrolysis of gum tragacanth (*Astragalus gummifer* Lab.) affords valuable index of purity of this commodity when compared with results obtained by similar treatment of so-called "Indian gum" (*Cochlospermum gossypium* D. C. and *Sterculia urens* Roxb.).

Treat 1 g whole or powd. sample in 700 ml round-bottom, long-neck flask in cold with 100 ml H_2O and 5 ml H_3PO_4 several hr, or until gum is completely swollen. Boil gently 2 hr under reflux condenser. Very small quantity of cellulose substance remains undissolved. Tragacanth yields practically colorless soln. Indian gum gives pink or rose soln. This reaction may be used as preliminary test for detection of Indian gum.

Distill hydrolyzed product with steam, using scrubber, Fig. 66, page 521, to connect distn flask with condenser. Continue distn until distillate totals 600 ml and acid residue is ca 20 ml. To avoid scorching residue, do not conc. contents of distg flask to <20 ml. Titr. distillate with 0.1*N* NaOH, using 10 drops phthln. Correct result by blank detn and express as "volatile acidity," ml 0.1*N* NaOH required to neutralize volatile (acetic) acid obtained.

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(*130*) Ibid. **22,** 757(1939).
(*131*) Ibid. **41,** 493(1958).
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(*134*) Ibid. **7,** 20(1923).
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(*137*) Ibid. **41,** 504(1958).
(*138*) Ibid. **10,** 383(1927); **11,** 362(1928).
(*139*) Ibid. **18,** 464(1935); **20,** 592(1937).
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(*145*) Ibid. **13,** 332(1930); **14,** 330(1931); **15,** 418(1932).
(*146*) Ibid. **37,** 697(1954).
(*147*) Ibid. **22,** 748(1939).
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(*149*) Ibid. **37,** 685(1954).
(*150*) Ibid. **19,** 535(1936); **21,** 557(1938); **22,** 730(1939).
(*151*) Ibid. **12,** 264(1929); **14,** 360(1931); **22,** 761(1939).
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(*154*) Ibid. **42,** 466(1959).

(*155*) Ibid. **39,** 639(1956).
(*156*) Ibid. **31,** 547(1948); **33,** 203(1950).
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(*165*) Ibid. **21,** 552(1938).
(*166*) Ibid. **17,** 451(1934); **18,** 519(1935).
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(*168*) Ibid. **32,** 549(1949).
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(*170*) Ibid. **9,** 288(1926).
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(*177*) Ibid. **15,** 448(1932); **16,** 375(1933).
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(*183*) J. Pharm. Chim., 8th ser. **16,** 49(1932); J. Assoc. Offic. Agr. Chemists **18,** 526(1935).
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33. Drugs in Feeds

(Medicated feeds may deteriorate under improper storage conditions. When possible, use reasonably fresh samples, store them in the cold, and grind just before analysis.)

Total Arsenic (*1*)—Official

33.001 REAGENTS

(**a**) *Arsenic trioxide.*—NBS As_2O_3 Reference Standard or equiv.

(**b**) *Magnesium oxide-magnesium nitrate slurry.*—Suspend 75 g MgO and 105 g $Mg(NO_3)_2.6H_2O$ in enough H_2O to make 1 L. Agitate vigorously before addn to sample. (Freshly prepd slurry gives ash which is easily disturbed by air currents.)

(**c**) *Stannous chloride soln.*—**24.001**(**a**). Effective as long as it discharges yellow color in sample ext.

(**d**) *Absorbing soln.*—Transfer with graduated cylinder 25 ml 1.5% $HgCl_2$ soln, and with pipet 3.75 ml 6*N* H_2SO_4 and 3.75 ml 0.03*N* $KMnO_4$ into 250 ml graduated cylinder. Dil. to 250 ml with H_2O and mix. Prep. fresh daily.

(**e**) *Ammonium molybdate reagent.*—Dissolve 1 g $(NH_4)_2MoO_4$ in 100 ml 5.4*N* H_2SO_4. Soln keeps several weeks. (Prep. 5.4*N* H_2SO_4 by dilg 6*N* 9+1.)

(**f**) *Hydrazine sulfate reagent.*—0.15%. Dissolve 0.15 g $N_2H_4.H_2SO_4$ in 100 ml H_2O. Soln keeps several weeks.

33.002 APPARATUS

(**a**) *Evaporating dishes.*—70 ml capacity; Coors No. 430, size 00A, or equiv.

(**b**) *Arsine evolution apparatus.*—Bend 6 mm i.d. glass tubing at 120° angle ca 4″ from one end and at 60° angle ca 6″ from other end. Plug shorter end with glass wool impregnated with satd $Pb(OAc)_2$ soln and insert in rubber stopper, placed in top of 125 ml erlenmeyer, so that end of tube projects just below stopper. Plug other end with unimpregnated glass wool and connect thru rubber tubing to glass tube, constricted at lower end, that reaches to bottom of 50 ml large neck vol. flask, or if preferred, 50 ml centrifuge tube, marked exactly at 50 ml and approx. at 20 ml.

33.003 PREPARATION OF SAMPLE SOLUTION

Weigh ground sample contg not >50 mmg As (unless aliquot is to be taken from digested soln) into 70 ml ashing dish. If >2.5 g sample is used, increase amount of slurry and size of ashing dish. Add ca 10 ml well-mixed slurry, **33.001**(**b**), and enough H_2O to permit thoro mixing with stirring rod. Rinse stirring rod, and dry sample at 100°. Ash 2–4 hr at 550–600°. (Slight C residue does not interfere. Use care to avoid loss of ash.)

Cool, and moisten residue with H_2O. Cover dish with watch glass and add ca 15 ml HCl (1+1). Let stand overnight, or heat on H_2O bath with agitation until ash dissolves. Filter thru Whatman No. 30 paper into 125 ml erlenmeyer. Rinse filter with enough hot H_2O, in several portions, to obtain ca 60 ml filtrate.

33.004 ARSINE EVOLUTION

Add ca 10 ml HCl, 2 ml KI soln, **24.001**(**c**), and 0.5 ml $SnCl_2$ soln, **33.001**(**c**). Swirl, heat in H_2O bath 5 min., and cool. Have all parts of evolution app. ready for immediate assembly, with ca 20 ml absorbing soln, **33.001**(**d**), in 50 ml vol. flask or centrifuge tube marked at 50 ml. Add 5–6 g Zn, **24.001**(**b**), to digested soln; quickly insert stopper contg glass tubing into the erlenmeyer and place delivery tube against bottom of vol. flask or centrifuge tube so that bubbles will be small. Use few drops of H_2O to test for leaks between rubber stopper and erlenmeyer. Connecting glass tube must be large enough so bubbles will not carry over Pb compounds from impregnated glass wool plug into absorption flask.

33.005 COLOR DEVELOPMENT

After 30 min. disconnect rubber tubing, leaving delivery tube in receiving vessel so that any Hg arsenide on tube will be exposed to color-developing reagents. Add 1.0 ml NH_4 molybdate reagent, **33.001**(**e**), and mix by forcing air thru delivery tube. Add 1.0 ml hydrazine sulfate reagent, **33.001**(**f**), and again mix. Heat in boiling H_2O bath 20 min. Rinse delivery tube with H_2O and remove. Cool to room temp., dil. to 50 ml, and mix. Filter thru tight glass wool plug in funnel or centrifuge. (Do not use filter paper, as color will be absorbed.) Read absorbance against H_2O at wavelength of max. absorption available in spectrophotometer used (750 mμ or above). Det. As content from std curve. As×2.90 = arsanilic acid; As×2.24 = arsenosobenzene; As×3.51 = 3-nitro-4-hydroxyphenylarsonic acid; As×2.91 = 4-nitrophenylarsonic acid.

33.006 PREPARATION OF STANDARD CURVE

Dissolve 0.660 g As_2O_3 in 25 ml 10% NaOH soln, dil. to 1 L with H_2O, and mix. Dil. 10 ml

aliquot to 1 L with H_2O (1 ml = 5 mmg As). Transfer 0, 2, 4, 6, 8, 10, 12, and 14 ml aliquots from buret into 125 ml erlenmeyers. Dil. each to ca 60 ml with H_2O and proceed as in **33.004.** Plot absorbance against mmg As.

p-Arsanilic Acid (*2*)—Official

(Applicable in absence of sulfonamides)

33.007 REAGENTS

Coupling reagent.—0.10% aq. soln of N-1-naphthylethylenediamine.2HCl. Prep. weekly and store in dark bottle.

33.008 DETERMINATION

Transfer 4.0 g freshly ground sample to 200 ml vol. flask, and add ca 80 ml H_2O and 4 ml 0.5*N* NaOH. Place flask on steam bath ca 5 min., swirling occasionally. Carefully add 20 ml HCl, mix, and cool to room temp. Dil. to vol. with H_2O, mix, pour into 250 ml beaker, add some Filter-Cel, and filter thru Whatman No. 42 paper (or equiv.), discarding first 5 ml.

Pipet 5 ml aliquots of clear filtrate into each of two 20×175 mm test tubes. To each tube add 2 ml *0.1% $NaNO_2$ soln,* mix, and let stand 5 min. Add 2 ml *0.5% NH_4 sulfamate soln* and let stand 2 min. Then add, to 1 tube only, 1 ml of the coupling reagent, mix, and let stand 10 min. before dilg both solns to vol. of 15 ml. Mix well, and det. absorbance against H_2O at 538 mμ in spectrophotometer or with 540 mμ filter in photometer. Subtract absorbance of blank from sample absorbance. Det. mmg arsanilic acid in aliquot (equiv. to 100 mg sample) from std curve.

33.009 PREPARATION OF STANDARD CURVE

Transfer 0.100 g pure *p-arsanilic acid* to 100 ml vol. flask, add ca 20 ml H_2O and 2 ml 0.5*N* NaOH, and dissolve. Dil. to vol. with H_2O and mix well. Transfer 10 ml to 100 ml vol. flask, dil. to vol. with H_2O, and mix well. Dil. 5 ml of this soln to 250 ml with H_2O in vol. flask, and mix well (1 ml = 2 mmg arsanilic acid). Pipet aliquots of 0, 2, 3, 5, and 8 ml of this std soln into 20×175 mm test tubes, add to each tube 1 ml HCl (1+1), and continue as in **33.008,** beginning "To each tube add 2 ml *0.1% $NaNO_2$ . . .*" Subtract blank absorbance from absorbance of stds and plot differences against 4, 6, 10, and 16 mmg arsanilic acid in aliquots.

Bithionol (2,2′-Thiobis[4,6-dichlorophenol]) (*3*)—First Action

33.010 REAGENTS

(**a**) *4-Aminoantipyrine soln.*—2% aq. soln. Stable at least 1 week. Development of yellow-green color does not affect usefulness of reagent. Reagent conforms to following specifications: m. p., 105–109°, corrected; purity by HNO_2 titrn, not <98%; sulfated ash, not >0.25%; color, colorless, or not more than light tan. (Available from Winthrop Labs., Inc., Special Chem. Dept., 1450 Broadway, New York 18, N.Y.)

(**b**) *Potassium ferricyanide soln.*—2% aq. soln. Stable at least 1 week.

(**c**) *Borax soln.*—Dissolve 50 g $Na_2B_4O_7.10H_2O$ in H_2O and dil. to 1 L.

(**d**) *Methanolic hydrochloric acid.*—0.1*N*. To 100 ml 1.0*N* HCl add 900 ml absolute MeOH.

(**e**) *Bithionol std soln.*—(*1*) *Primary soln.*—Dissolve 50.0 mg pure bithionol (available from Sterwin Chemicals, Inc., 1450 Broadway, New York 18, N.Y.) in 50.0 ml absolute methanol. Discard after 1 week at room temp. (*2*) *Working soln.*—20 mmg/ml. Dil. 2 ml of the primary soln to 100 ml with 5% borax soln. Use within 30 min. of prepn.

33.011 PREPARATION OF SAMPLE

Grind 0.5–1.0 lb sample and mix thoroly.

33.012 DETERMINATION

Transfer 2.0 g prepd sample contg ca 1 mg bithionol to 250 ml g-s. erlenmeyer. Pipet in 10 ml 0.1*N* HCl-MeOH, **33.010**(**d**), and 200 ml *n*-hexane. Shake mechanically 15 min.; then add 10 ml H_2O by pipet and shake addnl 15 min. Let stand until layers are well sepd (not >5–10 min. is necessary). Pipet 50 ml hexane layer into 100 ml g-s. graduated cylinder contg 10.0 ml 0.1*N* NaOH and shake 2 min. Clarify aq. soln by centrifuging, and continue assay within 30 min.

To series of five 60 ml separators contg 20 ml H_2O add following in order listed: working bithionol std soln, 0, 1.0, 2.0, 3.0, 0 ml; centrifuged alk. feed ext., 0, 0, 0, 0, 2.0 ml; 0.1*N* NaOH, 2.0, 2.0, 2.0, 2.0, 0 ml; 0.1*N* HCl, 2.0 ml to all; 5% borax soln, 3.0, 2.0, 1.0, 0.0, 3.0 ml; 2% aminoantipyrine soln, 0.4 ml to all; 2% $K_3Fe(CN)_6$ soln, 1.0 ml to all.

After swirling, add to each separator 15.0 ml 1-butanol. Stopper, and shake 1 min. Sep. and discard aq. layer. Swirl again and remove remaining H_2O. Decant 10 ml butanol ext. into tube graduated accurately at 10 ml. Add 1.0 ml acetone to each tube to clarify solns and mix by swirling. Read transmittance of solns in photometer, using ca 500 mμ filter, and calc. % bithionol in sample = mmg found/1000.

Diethylstilbestrol (*4*)—First Action

33.013 REAGENT

Diethylstilbestrol std soln.—55 mmg/ml. Prep. stock soln contg 0.55 mg USP Reference Standard

diethylstilbestrol/ml $CHCl_3$. Dil. 10 ml of this stock soln to 100 ml with $CHCl_3$.

33.014 APPARATUS

(a) *Lamp.*—Hanovia analytical model lamp, Cat. No. 7420, or SC-5010, or Hanovia Lethray J 24500 with 1-83-A-1 16″ Vycor lamp. (Hanovia Chemical and Manufacturing Co., Newark 5, N.J., or equiv.) Wattage of lamp and distance from cell should be such that peak absorption results with min. rise in temp. of soln, preferably not >1°.

(b) *Beckman DU spectrophotometer.*—Equipped with tungsten lamp or equiv. and matched 1 cm absorption cells. Cells must be silica or quartz, if used for irradiation.

(c) *Cells.*—Matched 1 cm quartz absorption cells or transparent quartz tubes, 16×150 mm (H. S. Martin Co., Cat. No. M-28580-2 to 4, or Microchemical Specialties Co., 1834 University Ave., Berkeley 3, Calif.) for irradiation.

33.015 STANDARDIZATION

Place quartz cells or tubes in suitable holder, rigidly fixed, so that cells or tubes are irradiated transversely and at fixed distance from lamp. (Do not irradiate from top.) Distance may be 6″ or whatever gives irradiation max. between ca 5–12 min. Cells or tubes must be clean and dry for each run. Det. optimum irradiation time by irradiation in quartz cells of ca 3 ml HOAc contg diethylstilbestrol at time intervals of 1 min. increments to find approx. irradiation max. Depending on source of irradiation, more exact irradiation time for max. absorbance may result with shorter time intervals.

Pipet 1 ml std soln into 100 ml beaker, evap. in air current, add 10 ml HOAc, stir, and transfer ca 3 ml portions to each of 2 or 3 cells for irradiation. Cool if necessary, and det. absorbance at 415 mμ as in **33.016**. Alternatively, use mixt. of alcohol and 0.1M KH_2PO_4 (1+1) as solvent.

Irradiation is critical and conditions must be carefully observed. Check frequently against std soln, and use exact same conditions for samples. Time of exposure may be longer with feed ext. than with pure diethylstilbestrol soln.

33.016 DETERMINATION

Weigh accurately 20 g of "40-mesh" sample, add 2–3 g fine asbestos, or *Dicalite* or other diatomaceous earth, mix well, transfer to extn thimble (33×94 mm), and place piece of absorbent cotton in top of thimble. Add to extn flask 140 ml solvent mixt. contg 7% v/v EtOH in $CHCl_3$, and ext. 16 hr or overnight at rate of at least 100 drops/min. in Soxhlet extractor. After extn, leave part of $CHCl_3$ in upper part of app. and transfer it to another flask. If insol. material is present in main ext., filter into 100 ml vol. flask (glass wool in funnel is suitable), rinse with rest of $CHCl_3$ from other flask, cool, dil. to vol. with $CHCl_3$, and mix.

Pipet 25 ml into 125 ml separator (aliquot A). Transfer another 25 ml into second 125 ml separator (aliquot B). Pipet into second separator 1.0 ml diethylstilbestrol std soln, equiv. to 55 mmg. Add 25 ml $CHCl_3$ and 25 ml 1N H_2SO_4 to each separator and wash by inverting funnel with rotary motion 6 times rather vigorously. Transfer lower $CHCl_3$ layer to another 125 ml separator, add 10 ml $CHCl_3$ to acid soln, and wash again as before. Combine $CHCl_3$ solns, discarding aq. soln.

Add 10 ml 1N NaOH, invert separator with rotary motion rather vigorously ca 12 times but not hard enough to cause serious emulsions. Drain $CHCl_3$ layer into another 125 ml separator. Again ext. this $CHCl_3$ with 10 ml 1N NaOH in same way. Discard $CHCl_3$ layer and combine alk. exts in separator.

To combined alk. exts add 5 ml $CHCl_3$, shake few sec., discard lower $CHCl_3$ layer, and repeat 3 or 4 times in same way until $CHCl_3$ exts are colorless. Transfer washed alk. ext. to 100 ml beaker. To this separator add 5 ml H_2O, shake, and add to combined alk. ext. Add 10 ml 2N H_3PO_4, cool to room temp., and adjust to pH 9.0±0.1 with 2N H_3PO_4. (CAUTION: Rinse separators that contained combined NaOH exts at least twice with H_2O to remove all alkalinity.)

Return pH-adjusted soln to original separator (alk.-free) from which it was taken, rinsing beakers with two 2 ml portions H_2O. Rinse beakers with 15 ml $CHCl_3$, shake 30–60 sec. carefully to avoid emulsions, transfer lower $CHCl_3$ layer to clean, dry separator, and ext. pH-adjusted soln twice more in same way with 15 ml portions of $CHCl_3$. Combine all $CHCl_3$ exts, add 25 ml H_2O, and shake briefly. Filter $CHCl_3$ ext. thru 30 ml fritted glass funnel (ca 20×100 mm), medium porosity, contg ¾″ of anhyd. Na_2SO_4, into 50 ml vol. flask. Wash the H_2O with 2–5 ml portions $CHCl_3$ and use to rinse Na_2SO_4 and funnel. Continue to rinse funnel with $CHCl_3$ to 50 ml mark. Mix, and pipet 25 ml aliquot into 150 ml beaker previously rinsed with $CHCl_3$. Evap. just to dryness on steam bath with aid of air current (manifold arrangement works well on series of samples). Evap. last of $CHCl_3$ with air current only.

Pipet 10 ml HOAc, or alcohol-0.1M K_2HPO_4 (1+1), into the beaker. Stir with glass rod to insure soln of diethylstilbestrol. Transfer ca 3 ml into each of 2 or 3 matched quartz cells (1 cm thickness). Read absorbance at 415 mμ before irradiation. Immediately place the cells in front of lamp, as in **33.015**, and irradiate under same conditions. Read absorbance. If quartz tubes are

used, readings before irradiation can be made on remaining 4 ml of soln for blank correction.

Calc. by increment method for each solvent. A = absorbance of sample after irradiation; A', before; B = absorbance of sample plus added diethylstilbestrol after irradiation; B', before.

$$\frac{A - A'}{(B - B') - (A - A')} \times 4.99$$
$$= \text{mg diethylstilbestrol/lb feed.}$$

Alternative calculations.—Prep. std curve from 27.5, 41.25, 55.0, 82.5, and 110 mmg diethylstilbestrol. After correcting for blank, det. mmg diethylstilbestrol from absorbance reading.

Mmg × 0.1815 = mg diethylstilbestrol/lb feed.

3,5-Dinitrobenzamide (*5*)—First Action

33.017 REAGENTS

(a) *Diethylamine reagent (DEA), aged.*—(1 year or older.) Fresh DEA may be artificially aged as follows: Place 1 L DEA in dry 2 L flask with 40 g Na or K fluosilicate. Connect flask to 24" bulb reflux condenser and reflux on sand bath 2–3 days in hood. When reagent is sufficiently "aged," 2 ml clear DEA added to 8 ml dimethylsulfoxide contg 50 mmg 3,5-dinitrobenzamide should develop max. color in ca 40 min. Absorbance as read on Beckman DU spectrophotometer at 560 mμ should be ca 0.375; on Klett-Summerson photoelec. colorimeter with No. 56 filter, ca 200. Reagent must be free from turbidity. Prep. new std curve for each batch of DEA.

(b) *3,5-Dinitrobenzamide (DNBA) std soln.*—Weigh 100 mg 3,5-DNBA into 100 ml flask and dil. to vol. with MeOH. (1 ml = 1 mg 3,5-DNBA.) Prep. working std soln by transferring 2.0 ml aliquot of this stock soln to 100 ml vol. flask and dilg to vol. with MeOH. (1 ml = 20 mmg 3,5-DNBA.)

33.018 PREPARATION OF STANDARD CURVE

Place 1.0, 2.0, 3.0, and 5.0 ml working std soln contg 20, 40, 60, and 100 mmg, resp., of 3,5-DNBA in 4 colorimeter tubes. Evap. to dryness in air current at 50°. Dissolve residue in 8 ml dimethylsulfoxide at 70°, cool, and add 2 ml DEA reagent. Place in dark at 20–25° and read after 60 min. Plot std curve, using absorbance readings as ordinate and concn as abscissa.

33.019 PREPARATION OF SAMPLE

Weigh 5.0 g feed, contg 0.025% 3,5-DNBA, into 100 ml vol. flask and dil. to vol. with MeOH. Shake frequently 20 min. and let stand 40 min. to permit feed particles to settle.

If feed contains 0.075% 3,5-DNBA, use 2 g finely ground feed; if 0.15%, use 1 g in 100 ml or 5 g in 500 ml MeOH. Prep. premixes by weighing appropriate sample and serially dilg MeOH ext.

33.020 DETERMINATION

Pipet 4 ml aliquot of ext. into g-s. test tube. Place tube in H_2O bath at 50° and evap. to dryness with air current directed onto surface of MeOH. Add 8 ml dimethylsulfoxide and heat to 70° to hasten soln, cool, and add 2 ml DEA reagent. Place in dark at 20–25° for 60 min. Det. absorbance at 560 mμ in Beckman DU spectrophotometer, Klett-Summerson photoelec. colorimeter with No. 56 filter, or similar instrument, against dimethylsulfoxide as reference.

Det. amount of 3,5-DNBA in tube from std curve.

% 3,5-DNBA in feed

$$= \frac{\text{mmg 3,5-DNBA in tube} \times 25 \times 100}{5{,}000{,}000};$$

or: mmg 3,5-DNBA in tube × 5 = mmg 3,5-DNBA/g of feed or ppm.

Enheptin ® (2-Amino-5-Nitrothiazole) (*6*)—Official

33.021 REAGENTS

(a) *Borate buffer.*—pH 9.0. Dil. 50.0 ml H_3BO_3-KCl soln, **13.023(c)**, and 21.40 ml 0.2*M* NaOH, **13.023(d)**, to 200 ml with H_2O.

(b) *Sodium hydrosulfite soln.*—Prep. 1% soln of Na hydrosulfite in borate buffer, (a), and use within 5 min. of prepn.

(c) *Enheptin reference std.*—Available from Lederle Laboratory Division, American Cyanamid Co., 30 Rockefeller Plaza, New York 20, N.Y.

33.022 DETERMINATION

Transfer 2 g ground feed to 50 ml wide-mouth vol. flask, add 10 ml acetone, and let stand 2 min., swirling occasionally. Dil. to vol. with H_2O, mix, and filter immediately thru *coarse* paper. Transfer 25 ml aliquot to 50 ml vol. flask, add 15 ml 5% NH_4Cl soln, and mix. Dil. to vol. with H_2O, mix, and filter thru Whatman No. 42 paper (or equiv.), discarding first 10 ml filtrate.

Place 4 ml aliquot in each of 2 small beakers. To first, add 0.5 ml of the freshly prepd Na hydrosulfite soln. Dil. contents of both beakers to 10 ml and immediately read both solns on spectrophotometer against H_2O at 388.5 mμ. Subtract absorbance of reduced soln from that of unreduced soln. Read from std curve mmg Enheptin corresponding to this difference.

Mmg Enheptin × 0.00125 = % Enheptin in sample.

33.023 PREPARATION OF STANDARD CURVE

Dissolve 100 mg recrystd 2-amino-5-nitrothiazole in 100 ml acetone and dil. to 1 L with H_2O. Transfer aliquots of 4, 8, 12, 16, and 20 ml to 100 ml vol. flasks and dil. to vol. with H_2O. Treat 5 ml aliquots of each diln as above, and read absorbance of unreduced soln against reduced soln as blank, obtaining readings corresponding to 20, 40, 60, 80, and 100 mmg.

Furazolidone (N-[5-nitro-2-furfurylidene]-3-amino-2-oxazolidone) and Bifuran (Mixture of Furazolidone and Nitrofurazone) (7)—First Action

33.024 REAGENTS

(a) *Phenylhydrazine hydrochloride soln.*—Dissolve 0.5 g phenylhydrazine.HCl in 50 ml H_2O. Prep. fresh daily. Mix equal vol. of this soln with HCl.

(b) *Furazolidone std soln.*—Weigh 110 mg cryst. furazolidone std (available from Hess & Clark, Inc., Ashland, Ohio) into 100 ml vol. flask, dil. to vol. with dimethylformamide, and mix. This soln is stable for several months when completely protected from light. For feeds contg 0.011% furazolidone, pipet 1 ml soln into 100 ml vol. flask, add 50 ml dimethylformamide, and dil. to vol. with H_2O. For feeds contg 0.00275% furazolidone, pipet 25 ml into 100 ml vol. flask and dil. to vol. with dimethylformamide. Pipet 1 ml of this soln into 100 ml vol. flask, add 50 ml dimethylformamide, and dil. to vol. with H_2O.

(c) *Bifuran std soln.*—Weigh 112 mg pure nitrofurazone (available from Hess & Clark, Inc., Ashland, Ohio) and 16.5 mg pure furazolidone into 100 ml vol. flask. (Alternatively, as practical std, 1 g bifuran may be used instead of the pure nitrofurans.) Mix and dil. to vol. with dimethylformamide. This soln is stable for several months when completely protected from light. For feeds contg 0.0064% total nitrofurans, pipet 1 ml soln into 200 ml vol. flask, add 100 ml dimethylformamide, and dil. to vol. with H_2O.

(d) *Absorbent.*—To 100 parts Merck Al_2O_3, chromatographic grade, in screw cap bottle, add 4 parts $Mg(OH)_2$, shake until thoroly mixed, then add 5 parts H_2O, and mix until all lumps disappear. Store in tightly sealed container.

33.025 DETERMINATION

Grind coarse or pelleted feeds to "20 mesh" thru cutting type mill such as Wiley Intermediate. Finer feeds need not be ground. Weigh 10 g sample into 125 ml erlenmeyer, add exactly 50 ml dimethylformamide, stopper loosely, and place in boiling H_2O bath 5 min. Shake on mechanical shaker 10 min. and filter thru rapid paper. To 25 ml filtrate add 25 ml H_2O and mix.

Prep. ca 20 mm diam. absorption column, contg Al_2O_3-$Mg(OH)_2$ absorbent, to height of 5 cm. Pass the 50% dimethylformamide sample soln thru column, discarding first 3 ml eluate. Pipet 5 ml aliquots of eluate into each of 2 numbered test tubes. Protect one tube from light. To other tube, add 2 drops *freshly prepd 2% soln of Na hydrosulfite* and let stand 20 min., shaking at ca 5 min. intervals. Treat 5 ml aliquots of dild std soln in exactly same manner.

Pipet 5 ml phenylhydrazine.HCl soln into each of the numbered test tubes contg samples and stds. Mix and place tubes in 70° H_2O bath 25 min.; cool in 15° H_2O bath 5 min. Add exactly 10 ml toluene to each tube, stopper, and shake vigorously 40 times. Centrifuge or filter toluene soln directly into absorption cell thru cotton wad inserted in stem of small funnel. Read absorbance (A) of solns at 440 mμ.

$$[(A_{samp.} - A_{red.\ samp.}) \times 0.011 \text{ (or } 0.00275)]/(A_{std} - A_{red.\ std}) = \%\text{ furazolidone.}$$

$$[(A_{samp.} - A_{red.\ samp.}) \times 0.0064]/(A_{std} - A_{red.\ std}) = \%\text{ total nitrofurans (bifuran).}$$

Nitrofurazone (5-Nitro-2-furaldehyde semicarbazone) (8)—First Action

33.026 REAGENTS

(a) *Phenylhydrazine hydrochloride soln.*—Dissolve 0.5 g phenylhydrazine.HCl in 67 ml H_2O. Prep. fresh for each set of samples. Mix equal vol. of this soln with 5N HCl.

(b) *Nitrofurazone std soln.*—Weigh 110 mg cryst. nitrofurazone (available from Hess & Clark, Inc., Ashland, Ohio) into 100 ml vol. flask, dil. to vol. with dimethylformamide, and mix. Soln is stable 1 week when completely protected from light. Prep. stds corresponding to label declaration. Pipet 25 ml soln into 50 ml vol. flask and dil. to vol. with dimethylformamide (Soln A). For feeds contg 0.0055% nitrofurazone: Pipet 1 ml soln A into 100 ml vol. flask and dil. to vol. with dimethylformamide. For feeds contg 0.011%: Pipet 1 ml soln A into 50 ml vol. flask and dil. to vol. with dimethylformamide.

33.027 DETERMINATION

Grind coarse or pelleted feeds to "20 mesh" thru cutting type mill such as Wiley Intermediate. Feeds of finer texture need not be ground.

Remove most of interfering feed colors by extg with *Skellysolve B* or *hexane*, in which nitrofurans are insol. Prep. $6 \times \frac{3}{4}''$ tube (test tube with small hole ca 5 mm diam. blown in bottom is satisfactory) by inserting pledget of cotton to form pad ca 5 mm thick when firmly compressed, then 5 mm layer of Hyflo Super-Cel, followed by 10 g

feed sample, tamped uniformly. Finally tamp another pledget of cotton on top of feed.

Wash column with four 15 ml portions Skellysolve B heated to near boiling. Let solvent pass thru column by gravity; if more than 10 min. is required, apply gentle suction. Insert tube into adapters and suck air thru until sample is completely dry as indicated by tube returning to room temp. Transfer sample together with pads to 125 ml flask by inverting tube and pushing rod thru hole in bottom. Add exactly 100 ml dimethylformamide, stopper, place on mechanical shaker 10 min., and filter thru rapid paper. (NOTE: If feed was pelleted, place flask in boiling H_2O bath until temp. of solvent reaches 92°, avoiding overheating with loss of solvent, and place on shaker as above.)

To 10 ml filtrate add exactly 10 ml H_2O and mix; pipet 5 ml aliquots of the dild soln into each of 2 numbered test tubes. Protect 1 tube from light; to other add 5–10 mg *Na hydrosulfite* and let stand 20 min., shaking at ca 5 min. intervals. Treat 10 ml of the dil. std soln in exactly same manner.

Pipet 5 ml acid-phenylhydrazine soln into each of the numbered test tubes contg samples and stds. Mix, and place tubes in H_2O bath 25 min. at 70°. Cool in 15° H_2O bath 5 min. Add exactly 10 ml toluene to each tube, stopper, and shake vigorously 40 times. Centrifuge or filter toluene soln directly into absorption cell thru cotton wad inserted in stem of small funnel. Read absorbance of solns at 440 mμ.

% Nitrofurazone $= (A_{\text{samp.}} - A_{\text{red. samp.}}) \times 0.0055$ (or $0.011)/(A_{\text{std}} - A_{\text{red. std}})$.

Glycarbylamide (4,5-Imidazoledicarboxamide) (*9*)—First Action

33.028 PRINCIPLES

Glycarbylamide is extd from feed with dimethylformamide. The ext. is purified by chromatography on Al_2O_3 and anion exchange resin and traces of residual impurities are oxidized with Br. Absorbance of glycarbylamide is measured at 283 mμ in alk. soln against portion of same soln from which glycarbylamide has been removed by adsorption on HgO.

33.029 REAGENTS

(**a**) *Dilute hydrochloric acid.*—Dil. 100 ml HCl to 1 L with H_2O.

(**b**) *Bromine soln.*—Dissolve 1.5 g $KBrO_3$ and 7.5 g KBr in H_2O and dil. to 250 ml.

(**c**) *Alkali soln.*—Dissolve 20 g Na metaborate ($NaBO_2.4H_2O$) and 45 g NaOH in enough H_2O to make 200 ml. Store in polyethylene bottle.

(**d**) *Sodium bisulfite soln.*—2% aq. soln. Prep. fresh.

(**e**) *Cyanide soln.*—Dissolve 1 g KCN in 100 ml 1*N* NaOH. POISON: *Handle with care.*

(**f**) *Aluminum oxide.*—Reagent grade for chromatography and conforming to following pH test: Transfer 10 g Al_2O_3 to 250 ml g-s. flask, add 100 ml H_2O, and shake vigorously at least 2 min. Let settle, decant soln, and det. pH electrometrically. pH should be 10.0–10.5.

(**g**) *Amberlite IRA-400.*—(Available from Rohm and Haas, Philadelphia, Pa.) Regenerate resin before use as follows: Place ca 200 ml resin in large glass column ca 2–3″ diam. and wash successively with ca 250 ml each of H_2O, 10% NaOH soln, H_2O, 10% HCl, H_2O, and 10% NaOH soln. Finally wash with H_2O until eluate is neutral to indicator paper. Store under H_2O in polyethylene bottles.

(**h**) *Mercuric oxide, red.*—Fine powder. Grind in mortar if necessary.

(**i**) *Glycarbylamide std solns.*—(1) *Stock soln.*—0.320 mg/ml. Weigh 32.0 mg glycarbylamide reference std (available from Merck & Co., Inc., Rahway, N. J.) into 100 ml vol. flask and dil. to mark with dimethylformamide. (2) *Working soln.*—6.4 mmg/ml. Dil. 5 ml stock std soln to 250 ml with dimethylformamide.

33.030 COLUMNS FOR CHROMATOGRAPHY

(**a**) *Aluminum oxide column.*—Use 50 cm glass tube, 22 mm i.d., constricted at one end. Place glass wool plug in constricted end and add 15 g Al_2O_3. Pack by gentle tapping on side of tube. Wash column with 25 ml dimethylformamide and let solvent drain to 1–2 cm above bed level before adding sample to column. Prep. sep. column for each sample and std.

(**b**) *IRA-400 column.*—Prep. column from 24 cm glass tube, 9 mm i.d. Seal upper end to reservoir of 8 cm of 5 cm o.d. tubing. Constrict lower end to hold glass wool plug and attach to piece of polyethylene tubing with screw clamp. Mark column at 4″ above glass wool plug. Add H_2O slurry of resin to column until, after letting H_2O drain, resin level reaches mark. Backwash resin bed by attaching source of H_2O to bottom of column, washing resin into reservoir, and letting it settle into column with no flow thru column. Then let H_2O drain to ca 1 cm above top of resin bed which should be at 4″ mark on column. Prep. sep. column for each sample and std.

33.031 EXTRACTION

Accurately weigh quantity of ground feed (not >25 g) contg ca 1.2 mg glycarbylamide (20 g for 0.006%) and transfer to 500 ml g-s. flask. Add 200.0 ml dimethylformamide and stopper. Stir vigorously 1 hr with magnetic stirrer. Transfer suspension to centrifuge tube and centrifuge 3–5 min. Alternatively, add 5–10 g Super-Cel or similar

filter aid to suspension and filter thru Whatman No. 42 paper on büchner.

33.032 CHROMATOGRAPHY

Pipet 100 ml clear filtrate onto Al_2O_3 column and let it pass thru column by gravity. Wash column with two 15 ml portions dimethylformamide followed by two 25 ml portions anhyd. MeOH. Discard washings.

Elute column with four 50 ml portions H_2O, letting H_2O pass thru column by gravity. Collect eluate in suitable container. Pass entire eluate thru Amberlite IRA-400 column at rate of 2–3 ml/min. Do not permit liquid level to drain into resin bed at any time. Wash column with 10, 20, and 20 ml portions H_2O, rinsing previous container, and drain at flow rate of 3 ml/min. Discard eluate and washings.

Elute column with 15 ml portions dil. HCl at ca 1–2 ml/min. (Do not exceed rate of 2 ml/min. or low results may be obtained.) Reject first 3 ml of eluate and collect 50 ml in vol. flask. Stopper and mix thoroly.

33.033 DETERMINATION

Pipet 25 ml of eluate into 50 ml g-s. flask, add 1.0 ml of the Br soln, mix, and let stand 3 min. Add 1.0 ml 2% $NaHSO_3$ soln, mix, and let stand 3 min. Finally add 5.0 ml of the alk. soln and mix carefully.

Pipet 10.0 ml of this soln into clean large test tube marked A. To remainder of soln add 1.0 g of the HgO, stopper, and shake vigorously 10 min., preferably on shaking machine. Transfer to centrifuge tube and centrifuge. Transfer 10.0 ml clear supernatant to clean large test tube marked R. Add 1.0 ml KCN soln to each tube, A and R, and mix. Soln R should not be unnecessarily exposed to light and absorbance measurement should be completed rapidly, preferably within 3 min. after addn of KCN soln. Det. absorbance of soln A against soln R in spectrophotometer at 283 mμ in 1 cm silica cell. Det. cell corrections, using reference soln R in both cells.

Prep. std by transferring 100.0 ml working std soln onto freshly prepd Al_2O_3 column and proceeding exactly as for sample.

% Glycarbylamide in feed $= 1.28AC/A'W$, where A = absorbance sample, A' = absorbance std, C = mg glycarbylamide in final aliquot of std (0.100 mg), and W = wt original sample in g.

Nicarbazin (4,4′-Dinitrocarbanilide.2-hydroxy-4,6-dimethylpyrimidine) (*10*)—Official

33.034 REAGENTS

(a) *Dimethylformamide.*—Reagent grade.

(b) *Aluminum oxide.*—Reagent grade suitable for chromatography and conforming to following pH test: Transfer 10 g sample to 250 ml g-s. erlenmeyer, add 100 ml H_2O, and shake vigorously at least 2 min. Let stand until insol. matter settles, decant soln, and det. pH electrometrically. pH should be 10.0–10.5.

(c) *Alcohol.*—Formulas SDA Nos. 2B, 3A, or 30 may be used.

(d) *Alcoholic sodium hydroxide soln.*—Dil. 2.0 ml clear 50% NaOH soln, **42.031(b)**, to 100 ml with alcohol. Centrifuge in stoppered tube. Prep. fresh daily.

(e) *Nicarbazin std stock soln.*—Weigh 25.0 mg nicarbazin reference std (available from Merck & Co., Rahway, N. J.) into 500 ml vol. flask, and dissolve in ca 150 ml dimethylformamide with aid of gentle heat. Cool, dil. to vol. with dimethylformamide, and mix well. Store protected from light.

(f) *Nicarbazin working std soln.*—Transfer 25.0 ml std stock soln to 100 ml vol. flask and dil. to vol. with dimethylformamide. Mix well. (1 ml = 12.5 mmg nicarbazin.)

33.035 PREPARATION OF COLUMN

Use glass tube 22 mm i.d., ca 50 cm long, constricted at lower end. Place plug of glass wool in constricted end and add 30 g of the Al_2O_3 in 3 portions. Tamp down each portion with glass rod while applying gentle suction. Wash column with 25 ml dimethylformamide, draining to point 1–2 cm above bed level before adding sample to column. Prep. column for each sample and std.

Never let column run dry; keep head of liquid at all times.

33.036 PREPARATION OF SAMPLE

Weigh 10.0 g sample into 250 ml erlenmeyer and add 100.0 ml dimethylformamide. Heat *just to boiling* on hot plate in hood with intermittent stirring. Cool to room temp. by immersing in H_2O bath. Decant supernatant into centrifuge tubes and centrifuge 3 min.

33.037 DETERMINATION

Pipet 25.0 ml clear ext. onto column and let pass thru column with aid of gentle suction. Wash column with three 10 ml portions dimethylformamide and reject washings. Elute with nine 5 ml portions alcohol, discarding first 15 ml eluate and collecting next 25 ml eluate in 1×8″ tube. Transfer eluate quantitatively into 50 ml vol. flask and dil. to vol. with alcohol. Mix well.

Pipet 25.0 ml nicarbazin working std onto another column and proceed as for sample.

Pipet two 15.0 ml portions sample soln into sep. 25 ml vol. flasks. To one add 5.0 ml alc. NaOH soln and adjust vol. of both solns to 25 ml with alcohol. Read yellow color formed in first flask within 5 min. in spectrophotometer or

colorimeter at 430 mμ against second soln as blank. Calc. wt nicarbazin from std curve.

33.038 PREPARATION OF STANDARD CURVE

Pipet 10, 15, and 20 ml aliquots of chromatographed std soln into sep. 25 ml vol. flasks, add 5 ml alc. NaOH, and dil. to vol. with alcohol. Mix well. Measure absorbance within 5 min. at 430 mμ against alcohol.

Prep. std curve by plotting absorbance against mmg nicarbazin.

33.039 IDENTIFICATION TEST

Place alcohol in 1 cm quartz cell and clear chromatographed sample soln in matched cell. Det. absorbance at 2 mμ intervals from 340 to 349 mμ with Beckman Model DU spectrophotometer (or equiv.) at min. slit width. Absorption max. at 344 ± 4 mμ confirms presence of nicarbazin.

Nithiazide (1-Ethyl-3-(5-nitro-2-thiazolyl) urea) (*11*)—First Action

33.040 REAGENTS

(**a**) *Dimethylformamide.*—Reagent grade. If absorbance of reagent blank as detd in **33.043** is >0.050, purify as follows: Add 1 g activated charcoal, NF/100 ml dimethylformamide. Shake ca 2 min., and filter. Refilter if not clear.

(**b**) *Aluminum oxide.*—Reagent grade suitable for chromatography. Should pass following test: Shake vigorously at least 2 min. 10 g of the Al_2O_3 with 100 ml H_2O in 250 ml g-s. flask. Let settle, decant, and det. pH electrometrically. The pH should be 9.5–10.5. (Aluminum Oxide Merck Reagent Chromatographic 71707 is suitable.)

(**c**) *Sodium hydroxide soln.*—1.0*N* aq. soln.

(**d**) *Procaine soln.*—Dissolve 100 mg procaine .HCl, USP, in 70 ml H_2O, add 20.0 ml HCl, mix well, cool to room temp., dil. to 100 ml with H_2O, and mix well.

(**e**) *Coupling reagent.*—0.10% aq. soln of N-(1-naphthyl) ethylenediamine.2HCl. Prep. fresh daily.

(**f**) *Nithiazide std stock soln.*—0.400 mg/ml. Weigh 40.0 mg Nithiazide Reference std (available from Merck & Co., Rahway, N. J.) into 100 ml vol. flask and dissolve in dimethylformamide to make 100.0 ml. Protected from light, soln is stable ca 6 weeks.

(**g**) *Nithiazide working std soln.*—10 mmg/ml. Pipet 5.00 ml std stock soln into 200 ml vol. flask, dil. to mark with dimethylformamide, and mix well.

33.041 EXTRACTION

Weigh accurately quantity of ground sample (not >4 g) contg ca 0.5 mg nithiazide and transfer to 100 ml vol. flask. Add 50.00 ml dimethylformamide, loosely stopper flask, and heat 10 min. at 60–75° (not >75°) in H_2O bath or on steam bath, swirling frequently. Remove flask and shake 15 min. on shaking machine. Cool to room temp., transfer mixt. to 50 ml centrifuge tube, and centrifuge.

33.042 CHROMATOGRAPHY

(**a**) *Preparation of aluminum oxide.*—Transfer 200 g Al_2O_3 to 1 L beaker. Add 500 ml H_2O and agitate 5 min. with mechanical stirrer. Let settle 5 min. and decant off supernatant. Repeat washing with 2 addnl 500 ml portions H_2O and decant supernatant as completely as possible. Add 300 ml MeOH to Al_2O_3 and agitate ca 3 min. Filter thru büchner, continue to apply suction ca 5 min., and dry 4 hr at 110°. Store in tightly stoppered bottle.

(**b**) *Preparation of column.*—Constrict end of 40 cm length of 9–10 mm i.d. glass tubing by rotating in hot flame until opening is 4–5 mm. Insert small plug of Pyrex glass wool in lower end of tube and compress with glass rod to thickness ca 2–3 mm. Weigh 3.0 g prepd Al_2O_3 and add to column in 2 equal portions. Lightly tamp each portion with glass rod while applying gentle suction. Wash column with 10.0 ml dimethylformamide and drain liquid to ca 5 mm above bed level prior to adding sample soln to column. Do not permit column to run dry; keep 5 mm head of liquid at all times. Prep. column for each sample, std, and reagent blank.

(**c**) *Chromatography of the feed extract.*—Pipet 20.00 ml clear feed ext. onto column and let it pass thru column by gravity. Do not let column run dry; keep 5 mm head of liquid. Wash inner walls of column and Al_2O_3 with three 4.0 ml portions dimethylformamide added from pipet. Let final dimethylformamide wash drain thru column until no further liquid appears at tip of column. Dry tip of column with filter paper.

Elute column by gravity with 4 ml portions H_2O. Collect first 1.0 ml eluate in graduated cylinder and reject; then collect eluate in 25 ml vol. flask until liquid level is just below mark. Adjust with H_2O to mark, stopper, and mix well. (Required time for elution should be not >60 min.; appreciably longer time indicates improper column prepn.)

33.043 DETERMINATION

Pipet 10.0 ml clear eluate into 25 ml vol. flask. Add 5.0 ml 1*N* NaOH, mix well, and let stand 10 min. at 20–25°. Add 5.0 ml procaine soln, mix well, and let stand 2 min. at 20–25°; then add sufficient coupling reagent to reach 25 ml mark, stopper, mix, and let stand 15 min. (If colored soln is not clear, filter thru clean sintered glass

funnel of medium or fine porosity.) Det. absorbance, A, of purple red color, in 1 cm cell in suitable spectrophotometer or colorimeter at 540 mμ, against H_2O as reference.

Prep. reagent blank by transferring 20.0 ml dimethylformamide onto freshly prepd column and proceeding in exactly same manner as for sample.

Prep. std by transferring 20.0 ml nithiazide working std onto freshly prepd column and proceeding in exactly same manner as for sample.

% Nithiazide in feed $= 5C(A - B)/8W(A' - B)$, where A = absorbance of sample, A' = absorbance of std, B = absorbance of reagent blank, C = mg nithiazide in final aliquot of std (0.080 mg), and W = wt original sample in g.

Nitrophenide (m,m'-Dinitrodiphenyldisulfide) (*12*)—Official

(Applicable in presence of arsanilic acid)

33.044 REAGENT

(**a**) *Buffer soln.*—pH 6.6. Dissolve 41.29 g anhyd. Na_2HPO_4 in H_2O and dil. to 1 L. Dissolve 11.47 g citric acid.H_2O in H_2O and dil. to 1 L. Mix in equal proportions.

(**b**) *Nitrophenide reference std.*—Available from Lederle Laboratory Division, American Cyanamid Co., 30 Rockefeller Plaza, New York 20, N.Y.

33.045 DETERMINATION

Transfer 2 g ground sample to 300 ml erlenmeyer, add 0.5 g $Na_2S_2O_4$ and 50 ml of the buffer soln, and place in boiling H_2O bath 20 min. Remove flask, slowly add 10 ml HCl, and replace flask in boiling H_2O bath 5 min. (This heating destroys arsanilic acid and ppts colloidal S.) Remove flask, connect to compressed air or vac. manifold, and aerate vigorously 15 min. Transfer to 100 ml vol. flask, cool, dil. to vol. with H_2O, and mix. Filter thru Whatman No. 42 paper (or equiv.), discarding first 15 ml filtrate if turbid.

Pipet 5 ml portions of clear filtrate into each of two 50 ml beakers; to each add 2 ml *freshly prepd 0.1% $NaNO_2$ soln*. After 5 min., add 2 ml *0.50% aq. NH_4 sulfamate soln,* and let stand 2 min. Add 1 ml coupling reagent, **33.007,** to first beaker and 1 ml H_2O to second. Mix solns thoroly after adding each reagent. After 10 min., add 15 ml H_2O to each beaker and mix. Read absorbance of both solns against H_2O blank in spectrophotometer at 545 mμ. Subtract absorbance of feed blank from sample absorbance and det. quantity of nitrophenide from std curve. Divide by 1000 to obtain % nitrophenide.

33.046 PREPARATION OF STANDARD CURVE

Transfer 0.10 g pure nitrophenide to 100 ml vol. flask, dissolve in 50 ml acetone, and dil. to vol. with acetone. Pipet 10 ml aliquot into another 100 ml vol. flask and dil. to vol. with acetone. Pipet 2, 3, 4, 5, and 6 ml portions of this dild soln into sep. 100 ml vol. flasks and carefully evap. in gentle stream of air. To each flask add 0.5 g $Na_2S_2O_4$ and 50 ml of the buffer soln, and proceed as in **33.045,** aerating 20 min. Plot std curve representing 10, 15, 20, 25, and 30 mmg nitrophenide against absorbances.

NOTE: Detn may be performed in 100 ml vol. flasks, if care is taken to add HCl *slowly* and in *small portions* with constant swirling, after reduction, in order to avoid excessive foaming and loss of soln.

Phenothiazine (*13*)—Official

33.047 REAGENT

Phenothiazine std soln.—Dissolve 10 mg recrystd phenothiazine in 50 ml alcohol and dil. to 100 ml with alcohol. For working stds, dil. with equal vol. of alcohol. (1 ml dild soln = 50 mmg phenothiazine.) Use freshly prepd soln; alc. solns gradually develop rose tint within few hr.

33.048 DETERMINATION

Place 1 g ground sample in 100 ml vol. flask, add 50 ml alcohol, and heat on steam bath 15 min. Cool, dil. to vol. with alcohol, mix, and let settle (ca 15 min.) until supernatant is clear.

Place 2 ml aliquot in 25 ml vol. flask and add 10 ml alcohol. To flask add, in order given, 1 ml *1% alc. p-aminobenzoic acid,* 1 ml *aq. 2% $NaNO_2$,* and 1 ml HCl (1+3). Dil. to vol. with alcohol. Read absorbance of green color at 600 mμ in spectrophotometer against reagent blank. Det. quantity phenothiazine from std curve. % phenothiazine = mmg/200.

Prep. reference curve, using 1,2, and 3 ml dil. std soln, as above.

Reserpine (*14*)—First Action

33.049 REAGENTS

(**a**) *2,2,4-Trimethylpentane.*—Ext. with H_2SO_4 until exts are practically colorless and wash with H_2O until washings are neutral to litmus paper. Dry the trimethylpentane with silica gel and redistill, discarding forerun and residue, and collecting that portion that distills between 98.5–100°. Matheson Coleman and Bell Reagent or Phillips Spectro grade are satisfactory without further purification.

(**b**) *Citric acid soln.*—Dissolve 2.0 g citric acid .H_2O in H_2O, and dil. to 100 ml.

(**c**) *Sodium nitrite soln.*—Dissolve 0.3 g $NaNO_2$ in 50 ml H_2O, and dil. to 100 ml with MeOH. Prep. fresh daily.

(**d**) *Sodium bicarbonate soln.*—Dissolve 1.0 g $NaHCO_3$, and dil. to 100 ml.

(e) *Sulfamic acid soln.*—Dissolve 2.5 g sulfamic acid in H_2O, and dil. to 50 ml. Prep. fresh daily.

(f) *Reserpine std soln.*—5 mmg/ml. Dissolve 25 mg reserpine in $CHCl_3$, and dil. to 100 ml. Prep. working standard by dilg 2 ml stock soln to 100 ml with $CHCl_3$. Prep. fresh daily.

33.050 APPARATUS

(a) *Photofluorometer.*—Coleman Model 12C with B-1 filter (Corning 5874) and PC-1 filter (combination of Corning 3389 and 4308) or other suitable photofluorometer with photomultiplier tube filters.

(b) *Separators.*—500 ml and 125 ml, Squibb type with Teflon stopcocks (such as Lab Crest); Fischer and Porter Co., Hatboro, Pa., or equiv.

(c) *Funnel.*—Büchner type with fritted disk, medium porosity, 60 ml, such as Scientific Glass Apparatus Co., Bloomfield, N. J., catalog No. F-8155, or equiv.

(d) *Rotating vacuum evaporator.*

(e) *Filter bell.*—NYLAB (New York Laboratory Supply Co., Inc., New York, N. Y.) Catalog No. 35070, or equiv.

33.051 EXTRACTION

Grind pelletized feeds to "20 mesh" thru cutting type mill such as Wiley. Weigh accurately ca 25 g sample contg ca 50 mmg reserpine and transfer to fritted glass funnel, (c), contg pad of glass wool. Add ca 10 ml $CHCl_3$ (do not stir), collecting filtrate in 100 ml vol. flask by means of filter bell and mild vac. Continue extn with 10–15 ml portions $CHCl_3$ until total of ca 100 ml $CHCl_3$ collects, stirring feed in final extn. Dil. to vol. with $CHCl_3$, and mix well. Keep time of exposure of reserpine to $CHCl_3$ and light at min.

Pipet 40 ml $CHCl_3$ ext. to 500 ml separator and add 400 ml trimethylpentane. Ext. with eight 10 ml portions citric acid soln, avoiding emulsions by gentle shaking. Combine citric acid exts in 125 ml separator and ext. with one 20 ml and five 10 ml portions $CHCl_3$, avoiding emulsions by gentle shaking. Combine $CHCl_3$ exts in another 125 ml separator, add 15 ml $NaHCO_3$ soln, and shake 1 min. Let layers sep. ca 5 min., and drain $CHCl_3$ into 500 ml round-bottom flask. Wash $NaHCO_3$ soln with 10 ml $CHCl_3$, and add to $CHCl_3$ ext. Evap. $CHCl_3$ soln to ca 10 ml under vac. at temp. not $>60°$ in rotating vac. evaporator. Transfer $CHCl_3$ soln to 25 ml vol. flask, using exactly 10 ml MeOH to wash flask. Continue washing with 2–3 ml portions $CHCl_3$, dil. to vol. with $CHCl_3$, and mix well.

To 4 ml working std reserpine soln in 500 ml separator, add 35 ml $CHCl_3$ and 400 ml trimethylpentane, and mix well. Treat std similarly to sample as above beginning, "Ext. with eight 10 ml portions citric acid soln . . ."

33.052 DETERMINATION

Pipet two 5 ml portions of feed ext. and two 5 ml portions of std prepn into sep. 10 ml vol. flasks. To each soln add 2.0 ml MeOH; to one of flasks contg std prepn, and to one of flasks contg feed prepn, add 1.0 ml $NaNO_2$ soln and 6 drops HCl. To other flasks (blanks) add 6 drops HCl. Mix contents of each flask, and let stand at least 30 min., shaking occasionally. Add 0.5 ml sulfamic acid soln to each flask, dil. to vol. with MeOH, mix well, and let stand for 10 min., shaking occasionally.

Immediately before developing fluorescences, prep. unextd std by transferring 4 ml working std reserpine soln to 25 ml vol. flask contg 10 ml MeOH and dilg to vol. with $CHCl_3$. Pipet two 5 ml portions unextd std into sep. 10 ml vol. flasks, and treat similarly to feed prepn as above, beginning "To each soln add 2.0 ml MeOH; . . ." Adjust instrument to give galvanometer reading of 50 with unextd $NaNO_2$-treated std, and adjust galvanometer to zero reading with corresponding blank. Measure fluorescence of std prepn, S; feed prepn, F; and respective blanks, S_0 and F_0, in photofluorometer.

Calculate % reserpine $(C_{33}H_{40}N_2O_9) = 2.5 \times (F - F_0)/[500W(S - S_0)]$, where W is g feed sample.

33.053 Sulfaguanidine *(15)*—**First Action**

Weigh 1 g ground sample into 250 ml vol. flask, and add 100 ml H_2O and 2.5 ml 0.50N NaOH. Heat in H_2O bath 15 min. with occasional swirling, cool, dil. to vol., and mix well. Let material settle, pipet 25 ml into 100 ml vol. flask, add 10 ml *1.00% $ZnSO_4.7H_2O$ soln*, dil. to mark, mix well, and let stand 1 min. Filter thru 18.5 cm Whatman No. 2 paper, discarding first 10 ml filtrate. (Filtrate must be free of turbidity.)

Pipet 2 ml clear filtrate into 25 ml vol. flask; add 2.5 ml 0.50N HCl and 2 ml *0.1% $NaNO_2$ soln (prepd fresh daily)*. Let stand 3 min. Add 2 ml *0.50% NH_4 sulfamate soln* and wait addnl 2 min. Finally add 2 ml coupling reagent, **33.007**, and dil. to mark. Swirl flask after addn of each reagent. Prep. blank, using H_2O and same quantities of reagents dild to 25 ml. Shake vigorously. Measure absorbance of colored soln in spectrophotometer at 545 mμ against reagent blank, and det. quantity of sulfaguanidine present by reference to std curve. Mmg sulfaguanidine × 0.05 = % sulfaguanidine in sample.

Prep. std curve as follows: Dissolve 0.010 g pure sulfaguanidine in 2.5 ml 0.50N NaOH and 100 ml H_2O in 250 ml vol. flask by heating 15 min. in boiling H_2O bath. Cool, and dil. to vol. with H_2O.

Pipet 25 ml of this soln into 100 ml vol. flask, add 10 ml of the $ZnSO_4$ soln, dil. to vol., and filter (1 ml = 10 mmg sulfaguanidine). Pipet 1, 2, 3, and 4 ml portions of this dild soln (equiv. to 10, 20, 30, and 40 mmg sulfaguanidine, resp.) into sep. 25 ml vol. flasks, dil. to 10 ml with H_2O, and proceed as in second par. beginning "add 2.5 ml 0.50*N* HCl . . ." Plot absorbance against mmg sulfaguanidine.

33.054 Sulfaquinoxaline (*16*)—Official

Weigh 5 g ground sample into 250 ml vol. flask, add 150 ml H_2O and 5 ml 0.5*N* NaOH, and place in boiling H_2O bath 15 min. Remove, cool, dil. to vol. with H_2O, mix, and let settle. Transfer 50 ml supernatant to 100 ml vol. flask, add 3 ml HCl, and dil. to vol. Mix, and filter thru 18.5 cm Whatman No. 2 paper (or equiv.), discarding first 15 ml filtrate if turbid.

To 10 ml filtrate in each of two 50 ml beakers add 2 ml *freshly prepd 0.1% $NaNO_2$ soln* and let stand 3 min. Add 2 ml *0.5% NH_4 sulfamate soln* and let stand 2 min. Add 1 ml coupling reagent, **33.007,** to first beaker and 1 ml H_2O to second beaker. Mix solns thoroly after adding each reagent. After 10 min., read absorbance in spectrophotometer at 545 mμ. Subtract absorbance of feed blank from sample absorbance and det. quantity of sulfaquinoxaline from std curve. Divide by 1000 to obtain % sulfaquinoxaline.

Prep. std curve as follows: Dissolve 0.250 g pure sulfaquinoxaline in 5 ml 0.5*N* NaOH and 50 ml H_2O in 500 ml vol. flask, and dil. to vol. with H_2O. Pipet 5 ml aliquot of this soln into 100 ml vol. flask and dil. to vol. with H_2O. Pipet 2, 4, 6, 8, and 10 ml portions of this dild soln (equiv. to 50, 100, 150, 200, and 250 mmg sulfaquinoxaline, resp.) into sep. 100 ml vol. flasks, add 3 ml HCl to each flask, and dil. to vol. with H_2O. Treat 10 ml aliquots of these final dilns as in second par. Det. absorbances at 545 mμ against H_2O blank, and plot absorbances against mmg sulfaquinoxaline.

Sulfaquinoxaline and Arsanilic Acid (*17*)—Official

33.055 PREPARATION OF STANDARD CURVE

Prep. sep. curves for sulfaquinoxaline and arsanilic acid as in **33.054,** third par., using 0.250 g of each.

33.056 DETERMINATION

(a) *Total absorbance.*—Det. absorbance of sulfaquinoxaline and arsanilic acid as in **33.054,** par. 1 and 2. Let sample absorbance − feed absorbance = x.

(b) *Absorbance of arsanilic acid.*—Pipet 35 ml sample soln into separator; ext. with three 50 ml portions ether, discarding ether exts. Transfer aq. layer to erlenmeyer and aerate 10 min. to remove dissolved ether. Treat two 10 ml portions of this soln as in **33.054,** second par. Let difference between these 2 absorbances = y. From arsanilic acid std curve det. mmg arsanilic acid corresponding to y. Mmg arsanilic acid/1000 = % arsanilic acid in sample. Arsanilic acid × 1.101 = Na arsanilate ($NH_2C_6H_4AsO(OH)ONa$).

(c) *Absorbance of sulfaquinoxaline.*—Subtract y from x; from sulfaquinoxaline std curve det. mmg sulfaquinoxaline corresponding to this difference. This value/1000 = % sulfaquinoxaline in sample.

ANTIBIOTICS

Microbiological Methods (*18*)

33.057 CULTURE MEDIA

(a) *Agar medium A.*—Dissolve 6.0 g peptone, 4.0 g pancreatic digest of casein, 3.0 g yeast ext., 1.5 g beef ext., 1.0 g anhyd. dextrose, and 15 g agar in H_2O, and dil. to 1 L. Adjust with 1*N* NaOH or HCl (1+9) so that after sterilization for 30 min. at 121° pH is 6.5–6.6. (Difco Penassay Seed Agar and BBL Seed Agar have been found satisfactory.)

(b) *Agar medium B.*—Dissolve 6.0 g peptone, 3.0 g yeast ext., 1.5 g beef ext., 1.0 g anhyd. dextrose, and 15 g agar in H_2O, and dil. to 1 L. Adjust with 1*N* NaOH or HCl (1+9) so that after sterilization for 30 min. at 121° pH is 6.5–6.6. (Difco Yeast Beef Agar has been found satisfactory.)

(c) *Agar medium C.*—Dissolve 6.0 g peptone, 3.0 g yeast ext., 1.5 g beef ext., and 15 g agar in H_2O, and dil. to 1 L. Adjust with 1*N* NaOH or HCl (1+9) so that after sterilization 30 min. at 121° pH is 6.5–6.6. (Difco Penassay Base Agar and BBL Base Agar have been found satisfactory.)

(d) *Agar medium D.*—Use agar medium C adjusted with 1*N* NaOH or HCl (1+9) so that final pH is 5.6–5.7.

(e) *Agar medium E.*—Use agar medium C adjusted with 1*N* NaOH so that final pH is 8.1. (Difco Streptomycin Assay Agar and BBL Streptomycin Assay Agar with Yeast Extract have been found satisfactory.)

(f) *Agar medium F.*—Use agar medium A contg addnl 2.5 g agar/L and adjust to pH 6.6–6.8.

(g) *Agar medium G.*—Use broth medium B contg 20 g agar/L. (Difco and BBL Polymyxin Base Agar have been found satisfactory after addn of 0.4 ml 1% $MnCl_2$ soln.)

(h) *Broth medium A.*—Dissolve 5.0 g peptone, 1.5 g yeast ext., 1.5 g beef ext., 3.5 g NaCl, 1.0 g anhyd. dextrose, 3.68 g anhyd. K_2HPO_4, and 1.32 g anhyd. KH_2PO_4 in H_2O, and dil. to 1 L. Adjust with 1*N* NaOH or HCl (1+9) so that after sterilization for 30 min. at 121° pH is 6.95–7.05.

(Difco Penassay Broth and BBL Antibiotic Assay Broth have been found satisfactory.)

(i) *Broth medium B*.—Dissolve 17.0 g pancreatic digest of casein, 3 g papaic digest of soybean, 5 g NaCl, 2.5 g anhyd. K_2HPO_4, and 2.5 g anhyd. dextrose in H_2O and dil. to 1 L. Add 0.4 ml 1% $MnCl_2$ soln and adjust with 1*N* NaOH or HCl (1+9) so that after sterilization for 30 min. at 121° pH is 7.3. (BBL Trypticase Soy Broth has been found satisfactory after addn of $MnCl_2$ soln.)

33.058 REAGENTS

(a) *Phosphate-bicarbonate buffer*.—pH 8. Dissolve 16.73 g anhyd. K_2HPO_4, 0.523 g anhyd. KH_2PO_4, and 20 g $NaHCO_3$ in H_2O and dil. to 1 L.

(b) *Phosphate buffer*.—pH 7.0. Dissolve 13.6 g anhyd. K_2HPO_4 and 4.0 g anhyd. KH_2PO_4 in H_2O and dil. to 1 L.

(c) *Phosphate buffer*.—pH 6. Dissolve 8.0 g anhyd. KH_2PO_4 and 2.0 g anhyd. K_2HPO_4 in H_2O and dil. to 1 L.

(d) *Phosphate buffer*.—pH 4.5. Dissolve 13.6 g anhyd. KH_2PO_4 in H_2O and dil. to 1 L.

(e) *Pyridine-buffer soln*.—Mix 9 vols pyridine and 31 vols pH 6.0 buffer.

(f) *Acid-acetone*.—Mix 1 vol. 4*N* HCl, 13 vols acetone, and 6 vols H_2O.

(g) *Acid-methanol*.—Mix 1 vol. HCl and 50 vols MeOH.

33.059 APPARATUS

(Jars of high speed blenders, if used in place of mortars and pestles, must be cleaned with great care, after disassembling, to eliminate all traces of antibiotics. All equipment must be thoroly cleaned after use and heat-treated, if possible, 2 hr at 200°.)

(a) *Cylinders*.—Polished open stainless steel cylinders, 8±0.1 mm o.d., 6±0.1 mm i.d., and 10±0.1 mm high (obtainable from S & L Metal Products Corp., 25 Lafayette St., Brooklyn 1, N.Y.).

(b) *Petri dishes (plates)*.—20×100 mm with porcelain covers glazed on outside. (BBL Brewer cover lids with filter pad inserts have also been found satisfactory for absorbing H_2O of syneresis.)

33.060 STOCK CULTURES OF TEST ORGANISMS

For appropriate test organism designated below, prep. slant culture on 1 or more tubes of agar medium A. Incubate 18–24 hr at indicated temp. held constant to ±0.5°, and finally store in dark at ca 10°. Do not use if >2 weeks old.

(a) *Sarcina lutea*.—ATCC No. 9341. Incubate stock culture at 26°. Use for penicillin and oleandomycin assays.

(b) *Micrococcus flavus*.—ATCC No. 10240. Incubate stock culture at 32–35°. Use for bacitracin assay.

(c) *Sarcina subflava*.—ATCC No. 7468. Incubate stock culture at 32–35°. Use as alternative organism for bacitracin assay.

(d) *Bacillus cereus var. mycoides*.—ATCC No. 9634. Incubate stock culture at 30°. Use for chlortetracycline and oxytetracycline assays.

(e) *Bacillus subtilis*.—ATCC No. 6633. Incubate stock culture on agar medium F 16–18 hr at 37.5°. Use for hygromycin B assay.

33.061 PREPARATION OF STANDARD CURVE

Prep. std curve simultaneously with assay soln. Prep. concns of Reference Standard (described for each antibiotic). Use indicated concn as reference concn.

Prep. plates with appropriate base agar layer and appropriate seed agar layer (described for each antibiotic). Place 6 cylinders on each plate at ca 60° intervals on 2.8 cm radius. Fill 3 alternate cylinders with reference concn and other 3 cylinders with one of other concns of std. Use 3 plates for each concn required for std curve, except reference concn (total of 12 plates). Incubate plates 18–24 hr at appropriate temp., and read diams of zones of inhibition by means of mm ruler, calipers, or calibrated projection device. In each set of 3 plates average the 9 readings of the reference concn and the 9 readings of concn being tested. Av. of all 36 readings of reference concn from 12 plates is correction point for curve. Correct av. value obtained for each concn to appropriate figure if reference concn reading on that set of 3 plates were same as correction point.

For example, if in correcting second concn of std curve, av. of 36 readings of reference concn is 20.0 mm, and av. of 9 readings of reference concn of this set of 3 plates is 19.8 mm, correction is +0.2 mm. If av. reading of second concn on same 3 plates is 17.0 mm, corrected value is 17.2 mm. Plot corrected values, including correction point, on semilog graph paper, using logarithmic scale for concn and arithmetic scale for av. zone diams. Draw line of best fit by inspection or by following equations:

$$L = (3a + 2b + c - e)/5$$
$$H = (3e + 2d + c - a)/5$$

where L and H = calcd zone diams for low and high concns, resp., of std response line; a, b, c, d, and e = corrected av. zone diams for each concn on std response line.

Plot values for L and H and connect with straight line.

33.062 DETERMINATION

Use 3 plates for each assay soln. On each plate fill 3 alternate cylinders with reference concn and

fill other 3 cylinders with assay soln. Incubate plates 18–24 hr at appropriate temp. and read diam. of zones of inhibition. Average the 9 readings of reference concn and the 9 readings of assay soln. If assay soln gives larger average than reference concn, add difference between them to reference point on std curve. If assay soln gives smaller value than reference concn, subtract difference between them from reference point on std curve. Using corrected value of assay soln, det. quantity of antibiotic from std curve.

Penicillin—Official

33.063 STANDARD SOLUTIONS

(a) *Stock soln.*—Weigh accurately, in atmosphere of 50% relative humidity or less, ca 10 mg USP Penicillin Reference Standard. Dissolve in enough pH 6 buffer to give concn of exactly 100 units/ml. Store in dark at ca 10° not >2 days.

(b) *Std curve.*—Dil. appropriate aliquots of stock soln, (a), with enough pH 6 buffer to obtain concns of 0.0125, 0.025, 0.05, 0.10, 0.20 units/ml. Reference concn is 0.05 units/ml.

33.064 PLATES

(a) *Base layer.*—Add 10 ml melted agar medium A to sterile petri dishes, distribute evenly, and let harden on *perfectly level surface.*

(b) *Seed layer.*—Prep. inoculum for plates by one of following methods:

(1) *Broth culture.*—Wash growth from stock culture of *S. lutea* with ca 3 ml broth medium A, and transfer liquid to 100 ml broth medium A. Incubate 48 hr at ca 26° with continuous mechanical agitation. (This 48 hr culture is inoculum and it may be stored at ca 10° not >2 weeks.) Before actual assay, det. by prepn of trial plates the optimum concn (usually 2–5%) of inoculum to be added to agar medium B to obtain zones of inhibition of adequate size and sharpness.

(2) *Roux bottle culture.*—Wash growth from 24 hr slant culture with ca 3 ml broth medium A, and transfer liquid to surface of 300 ml agar medium A in Roux bottle. Spread suspension evenly over entire surface with aid of sterile glass beads. Incubate 24 hr at 26° and wash growth from agar surface with ca 15 ml 0.9% NaCl soln. Using photoelectric colorimeter and 18 mm diam. test tube as absorption cell, det. transmittance of 1:10 diln of this bulk suspension at 650 mμ, and, if necessary, adjust by diln so that 1:10 diln gives 10% transmittance. Use adjusted bulk suspension (not 1:10 diln) in prepg seed layer. (Bulk suspension may be stored at ca 10° several months.) Before actual assay, det. by prepn of trial plates the optimum concn (usually 0.3–0.5%) of inoculum to be added to agar medium B to obtain zones of inhibition of adequate size and sharpness.

Add appropriate amount of organism suspension to agar medium B previously melted and cooled to 48°. Mix thoroly and add 4.0 ml to each plate contg base layer. Distribute agar evenly by tilting plates from side to side with circular motion, and let harden. *Use plates same day prepd.*

33.065 ASSAY SOLUTION

(a) *Feed supplement concentrates.*—If supplement contains >100 g penicillin/lb, use 1 g sample; for lower concns, use 3 g sample. Add 25 ml aq. acetone (1+1) or pH 6 buffer (lower concns only), shake 2 min., let settle, and decant supernatant into 100 ml vol. flask. Repeat washing, shaking, and decanting with 25 and 50 ml portions aq. acetone (1+1) or pH 6 buffer. Combine decantates, dil. to 100 ml, and mix. Centrifuge 15 ml combined decantates ca 15 min. at 2000 rpm. Dil. aliquot of clear soln with enough pH 6 buffer to obtain estimated concn of 0.05 units/ml.

(b) *Mixed feeds.*—To 10 g sample add 35 ml pH 6 buffer or aq. acetone (1+1), shake 2 min., let settle, and decant supernatant into 100 ml vol. flask. Repeat washing, shaking, and decanting with 35 and 30 ml portions pH 6 buffer or aq. acetone (1+1). Combine decantates, dil. to 100 ml, and mix. Centrifuge 15 ml combined decantates ca 15 min. at 2000 rpm. Dil. aliquot of clear soln with enough pH 6 buffer to obtain estimated concn of 0.05 units/ml.

Designate final vol. obtained as assay soln.

33.066 ASSAY

Using penicillin std curve, assay soln, and plates, proceed as in **33.061–33.062**, incubating at 26°.

Bacitracin—Official

33.067 STANDARD SOLUTIONS

(a) *Stock soln.*—Dry ca 40 mg USP Bacitracin Reference Standard 3 hr at 60° in vac. oven at 5 mm pressure or less. Det. accurate dry wt and dissolve in enough pH 6 buffer to give concn of exactly 100 units/ml. Store in dark at ca 10° not >5 days.

(b) *Std curve.*—Dil. appropriate aliquots of stock soln, (a), with enough pH 6 buffer to obtain concns of 0.05, 0.10, 0.20, 0.40, and 0.80 units/ml. Reference concn is 0.20 units/ml.

33.068 PLATES

(a) *Base layer.*—Add 10 ml melted agar medium C to sterile petri dishes, distribute evenly, and let harden on *perfectly level surface.*

(b) *Seed layer.*—Wash growth from stock culture of *M. flavus* or *S. subflava* with ca 3 ml broth medium A and transfer liquid to surface of 300 ml agar medium A in Roux bottle. Spread suspension evenly over entire surface with aid of sterile

glass beads and incubate 18 hr at 32–35°. Wash growth from agar surface with ca 25 ml 0.9% NaCl soln. Using photoelectric colorimeter and 18 mm diam. test tube as absorption cell, det. transmittance of 1:50 diln of this bulk suspension at 650 mμ, and, if necessary, adjust by diln so that 1:50 diln gives 75% transmittance. (Adjusted bulk suspension, not 1:50 diln, is used in prepg seed layer.) Store adjusted bulk suspension at ca 10°. Before actual assay, det. by prepn of trial plates the optimum concn (usually 0.3–0.5%) of inoculum to be added to agar medium A to obtain zones of inhibition of adequate size and sharpness. For actual assay add appropriate amount of inoculum to agar medium A previously melted and cooled to 48°. Mix thoroly and add 4.0 ml to each of plates contg base layer. Distribute agar evenly by tilting plates from side to side with circular motion, and let harden. *Use plates same day prepd.*

33.069 ASSAY SOLUTION

Place 2 g feed supplement concentrate in 150 ml beaker, add 5 ml HCl (1+2.5), and stir 1 min. Check pH with test paper. If pH is >2, add more acid until pH 2 is reached. Add 45 ml pyridine-buffer soln and transfer mixt. to centrifuge tube. Shake well 5 min. and centrifuge ca 15 min. at 2000 rpm. Dil. aliquot of clear soln with enough pH 6 buffer to obtain estimated concn of 0.20 units/ml. Designate soln obtained as assay soln.

33.070 ASSAY

Using bacitracin std curve, assay soln, and plates, proceed as in **33.061–33.062,** incubating at 32–35°.

Chlortetracycline Hydrochloride—Official

33.071 STANDARD SOLUTIONS

(a) *Stock soln.*—Weigh accurately ca 40 mg USP Chlortetracycline Hydrochloride Reference Standard. Dissolve in enough 0.01*N* HCl to give concn of exactly 1000 mmg/ml. Store in dark at ca 10° not >5 days.

(b) *Std curve.*—Dil. appropriate aliquots of stock soln, (a), with enough pH 4.5 buffer to obtain concns of 0.01, 0.02, 0.04, 0.08, and 0.16 mmg/ml. Reference concn is 0.04 mmg/ml.

33.072 PLATES

(a) *Base layer.*—Add 6.0 ml melted agar medium D to sterile petri dishes, distribute evenly, and let harden on *perfectly level surface.*

(b) *Seed layer.*—Wash growth from stock culture of *B. cereus* var. *mycoides* with ca 3 ml sterile H_2O, transfer to surface of 300 ml agar medium A, and incubate 7 days at 30°. Wash growth from agar surface with ca 25 ml H_2O and heat suspension 30 min. at 65°. Centrifuge and decant. Wash residual spores 3 times with sterile H_2O, centrifuging and decanting each time. Discard H_2O washings. Heat residual spores 30 min. at 65° and resuspend in sterile H_2O. Keep this stock suspension at ca 10°. Before actual assay, det. by prepn of trial plates optimum concn (usually 0.03–0.10%) of inoculum to be added to agar medium D to obtain zones of inhibition with as little as 0.01 mmg chlortetracycline.HCl/ml. For actual assay add appropriate amount of inoculum to agar medium D previously melted and cooled to 48°. Mix thoroly and add 4.0 ml to each of plates contg base layer. Distribute agar evenly by tilting plates from side to side with circular motion, and let harden. *Use plates same day prepd.*

33.073 ASSAY SOLUTION

Using mortar and pestle or high-speed blender, grind 2 g feed supplement concentrate or 10 g mixed feed with 50 ml acid-acetone soln and transfer mixt. to 100 ml centrifuge tube. Wash mortar and pestle or blender jar with 50 ml acid-acetone and combine washings with ext. in centrifuge tube. Shake well 5 min. Centrifuge ca 15 min. at 2000 rpm. Remove 10 ml clear soln and adjust to pH 4.5 with 1*N* NaOH. Dil. adjusted soln with enough pH 4.5 buffer to obtain estimated concn of 0.04 mmg/ml. Designate soln so obtained as assay soln.

33.074 ASSAY

Using chlortetracycline.HCl std curve, assay soln, and plates, proceed as in **33.061–33.062,** incubating at 30°.

Oxytetracycline—Official

33.075 STANDARD SOLUTIONS

(a) *Stock soln.*—Weigh accurately ca 40 mg USP Oxytetracycline Reference Standard. Dissolve in enough 0.1*N* HCl to give concn of exactly 1000 mmg/ml. Store in dark at ca 10° not >5 days.

(b) *Std curve.*—Dil. appropriate aliquots of stock soln, (a), with enough pH 4.5 buffer to obtain concns of 0.05, 0.10, 0.20, 0.40, and 0.80 mmg/ml. Reference concn is 0.20 mmg/ml.

33.076 ASSAY SOLUTION

Proceed as in **33.073** except that where reference is made to acid-acetone, replace by acid-MeOH, and where reference is made to 0.04 mmg/ml, replace by 0.20 mmg/ml.

33.077 ASSAY

Using oxytetracycline std curve and assay soln, and chlortetracycline plates, proceed as in **33.061–33.062,** incubating at 30°.

Oleandomycin (*19*)—First Action

33.078 STANDARD SOLUTIONS

(**a**) *Heated feed extract.*—Distribute 20 g feed sample in 20×150 mm glass petri dish without cover and autoclave 90 min. at 20 lb pressure. Add same amount of pH 8 phosphate-bicarbonate buffer as used in prepn of Assay Soln, **33.079**, shake 45 min., decant, and collect supernatant. If necessary, filter or centrifuge to obtain clear soln.

(**b**) *Stock soln.*—Weigh accurately ca 40 mg Oleandomycin Chloroform Adduct Reference Standard (available from Biological Control Labs., Chas. Pfizer & Co., Brooklyn 6, N. Y.). Dissolve in ca 5 ml MeOH and dil. with enough pH 8 phosphate-bicarbonate buffer to give concn of 125 mmg/ml. Store in dark at ca 10° not >3 days.

(**c**) *Std curve.*—Dil. appropriate aliquots of stock soln with enough heated feed ext. to obtain concns of 0.045, 0.067, 0.10, 0.15, and 0.225 mmg/ml. Reference concn is 0.10 mmg/ml.

33.079 ASSAY SOLUTIONS

To 20 g mixed feed sample contg 1–20 ppm oleandomycin, add enough pH 8 phosphate-bicarbonate buffer to give estimated concn of 0.1 mmg/ml. Shake mechanically 45 min., let settle, decant, and collect supernatant as assay soln.

33.080 PLATES

Use single inoculated agar layer prepd by one of following methods:

(**a**) *Broth culture.*—Proceed as in **33.064(b)(1)**. Optimum concn is usually 0.5%.

(**b**) *Roux bottle culture.*—Proceed as in **33.064(b)(2)** and use adjusted bulk suspension (not 1:10 diln) in prepg inoculated agar. Optimum concn is usually 0.1%.

Add appropriate amount of organism suspension to agar medium B previously melted and cooled to 48°. Mix thoroly and add 10.0 ml to each petri dish. Distribute agar evenly by tilting plates from side to side with circular motion and let harden. *Use plates same day prepd.*

33.081 ASSAY

Using oleandomycin std curve, assay soln, and plates, proceed as in **33.061–33.062**, incubating at 37°.

Hygromycin B (*20*)—First Action

33.082 STANDARD SOLUTIONS

(**a**) *Stock soln.*—Weigh accurately amount of Hygromycin B Reference Standard (available from Microbiological Testing Dept., Eli Lilly and Co., Indianapolis, Ind.) contg 50,000 units, transfer to 50 ml vol. flask, and dil. to vol. with pH 7 phosphate buffer. Store in refrigerator not >2 weeks.

(**b**) *Std curve.*—Dil. appropriate aliquots of stock soln daily with enough pH 7 buffer soln to obtain concns of 15, 25, 50, and 75 units/ml. Reference concn is 25 units/ml.

33.083 PLATES

(**a**) *Base layer.*—Add 10 ml melted agar medium E to sterile petri dishes, distribute evenly, and let harden on perfectly level surface.

(**b**) *Seed layer.*—Wash growth from stock culture of *B. subtilis* with 10 ml broth medium B into 100 ml sterile broth medium B. Incubate 48 hr at 37° on mechanical shaker. Transfer 5 ml aliquot to surface of 300 ml agar medium G in Roux bottle. Incubate 7 days at 37°. Wash growth from agar surface with 75 ml sterile H_2O into 250 ml centrifuge bottle. Heat suspension 15–20 min. in H_2O bath at 65° to destroy vegetative cells. Centrifuge, decant, and resuspend cells in 75 ml H_2O. Repeat heating, centrifuging, and suspending twice, or until supernatant is clear. Final suspension is stock inoculum. Immediately before use inoculate 100 ml liquefied agar medium E cooled to ca 50° with 0.2 ml 1:10 diln of the stock inoculum.

33.084 ASSAY SOLUTION

Preparation of ion exchange resin column.—Slurry ca 1 lb Amberlite IRC-50 ion exchange resin with 2 L 1*N* H_2SO_4 3 hr. Wash until neutral with H_2O and gradually add solid LiOH with stirring until pH remains at 7–8. Let stand overnight and wash with H_2O at least 5 times. Neutralize to pH 7.0 with 1*N* H_3PO_4. Store under H_2O in glass container.

Place glass wool plug at bottom of 6 mm i.d. ×140 mm long column fitted with valve to control flow and 50 ml reservoir at top. Fill column with H_2O and add wet resin to within 20 mm of top of column. Drain H_2O to within 5 mm of resin surface. Wash with 25 ml sterile H_2O immediately before use.

Weigh 50 g sample of feed contg 6000–12,000 units/lb (30 g for 18,000–24,000 units/lb, 20 g for >24,000) into jar of high speed blender. Add 300 ml (500 for the higher potency feeds) pH 7 phosphate buffer soln and blend 5 min., operating blender from autotransformer set at 70. Centrifuge 10 min. at 2600 rpm. Adjust 125 ml supernatant to pH 5.0 with HCl (ca 0.5 ml). Add 50 ml $CHCl_3$ previously washed with pH 7.0 buffer, stopper, and shake thoroly. Centrifuge mixt. 10 min. at 2600 rpm. Remove aq. phase and adjust to pH 7.0 with 40% NaOH soln (ca 0.7 ml) and centrifuge.

Transfer 100 ml neutralized soln (75 ml if feed

contains 42,000 units/lb or more) to ion exchange column and adjust flow rate to 40 drops/min. Wash column with four 20 ml portions sterile H_2O. Elute hygromycin B with 50 ml NH_4OH (1+9) into 100 ml Pyrex beaker. Evap. to 3–5 ml and adjust to pH 7.0 with 1*N* HCl. Transfer to 10 ml vol. flask, dil. to mark with pH 7.0 phosphate buffer, and designate as assay soln. (Final concn should be ca 25 units/ml.)

33.085 ASSAY

Using hygromycin std curve, assay soln, and plates, proceed as in **33.061–33.062**, except use 6 plates for each concn required for std curve (total of 18 plates) and for each assay soln. The equations for L and H cannot be used. Incubate at 37°.

33.086 CALCULATION

Units/lb = [(units/ml assay soln)454 $\times a$]/[$b \times$ g sample $\times$ 10], where a = [ml pH 7 buffer (300 or 500) $\times$ ml neutralized soln put on column], and b = (125 + ml HCl + ml 40% NaOH).

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(*5*) Ibid. **42**, 239(1959).
(*6*) Ibid. **36**, 219(1953); **37**, 257(1954).
(*7*) Ibid. **40**, 463(1957); **41**, 333(1958); **43**, 310 (1960).
(*8*) Ibid. **40**, 467(1957); **41**, 51(1958).
(*9*) Ibid. **43**, 284(1960).
(*10*) Ibid. **39**, 321(1956); **40**, 469(1957); **41**, 52 (1958).
(*11*) Ibid. **43**, 295(1960).
(*12*) Ibid. **35**, 552(1952); **36**, 219(1953); **39**, 307(1956).
(*13*) Ibid. **41**, 338(1958); **42**, 254(1959).
(*14*) Ibid. **43**, 291(1960).
(*15*) Ibid. **34**, 559(1951).
(*16*) Ibid. **33**, 156(1950); **38**, 229(1955); **39**, 307 (1956).
(*17*) Ibid. **39**, 307(1956).
(*18*) Ibid. **40**, 857(1957).
(*19*) Ibid. **43**, 211(1960).
(*20*) Ibid. **43**, 213(1960).

34. Cosmetics

DEODORANTS AND ANTI-PERSPIRANTS

Aluminum and Zinc (*1*)—Official

34.001 REAGENTS

(**a**) *8-Hydroxyquinoline soln.*—Dissolve 5.0 g 8-hydroxyquinoline in 12 ml HOAc, dil. to 100 ml with H_2O, and filter if not clear. Prep. fresh soln at least every 2 weeks.

(**b**) *Ammonium acetate soln.*—Approx. 2*N*. Dissolve 150–160 g NH_4OAc in 1 L H_2O and filter if not clear.

(**c**) *Hydrochloric acid.*—Approx. 2*N*.

(**d**) *Ammonium hydroxide.*—Approx. 2*N*. (Quantity of NH_4OH required to neutralize 20 ml of the 2*N* HCl should be known to within ±2 ml.)

34.002 PREPARATION OF SAMPLE

(**a**) *Liquids.*—Dil. 5 ml sample to 250 ml with H_2O in vol. flask. If perfume oils sep., filter before taking aliquot for analysis.

(**b**) *Creams and pastes.*—Weigh accurately 2–3 g sample into 250 ml beaker. Add 5 ml HCl (HNO_3 if chlorides are to be detd) and ca 50 ml H_2O, and heat until oils liquefy and sep.; cool until oils solidify, and decant aq. layer thru fluted paper into 250 ml vol. flask. Return filter to original beaker and macerate thoroly. Repeat above extn twice, decant as before, and finally wash residue and paper thoroly with H_2O. (It is unnecessary to return filter paper to beaker after these extns.) Cool combined exts to room temp., dil. to mark with H_2O, and mix.

(**c**) *Solids.*—Weigh accurately 2–3 g sample into 250 ml beaker, add 5 ml HCl (HNO_3 if chlorides are to be detd) and ca 50 ml H_2O, and heat to boiling. Cool, and filter thru fluted paper into 250 ml vol. flask. If filtrate is cloudy, refilter thru fine quant. paper. Wash beaker and paper thoroly with H_2O. Cool flask and contents to room temp., dil. to mark with H_2O, and mix.

34.003 DETERMINATION

(**a**) *Interfering metals absent.*—Take aliquot of sample soln contg 12–25 mg Al or 20–60 mg Zn. Add 1–2 drops phthln, and then add the 2*N* NH_4OH until neutral or until faint permanent turbidity results. Add 5 ml HOAc (1+9), dil. to ca 100 ml, and heat to 70–90°. Add 10 ml of the 8-hydroxyquinoline soln and then slowly add the NH_4OAc soln until 20 ml (*see* NOTE) in excess of quantity required to produce permanent ppt has been added. If permanent ppt forms on addn of the 8-hydroxyquinoline, add only 20 ml of the NH_4OAc soln. Heat below b.p. 2–5 min. and set aside 30–60 min. (Moderate excess of 8-hydroxyquinoline is required for complete pptn. If enough reagent has been added, soln will be yellow at this point; if it is not, repeat detn, using larger quantity of 8-hydroxyquinoline soln.) Filter thru tared gooch, wash thoroly with H_2O, dry 1–2 hr at 130–140°, cool, and weigh. Dry again 30 min., cool, and weigh. Repeat to constant wt (±0.3 mg). (Alternatively, ppt may be dried overnight.)

$$\text{Wt ppt} \times 0.05872 = \text{Al};$$
$$\text{Wt ppt} \times 0.1849 = \text{Zn}$$

NOTE: Final pH of soln from which metals are pptd should be 4.9–5.1. Quantity of NH_4OAc soln required to produce this pH should be detd experimentally each time new set of reagents is prepd. If NH_4OAc is of usual purity, ca 20 ml of soln will be required.

(**b**) *In presence of magnesium.*—Ppt as in (**a**) and set aside ca 30 min. Decant most of liquid thru quant. paper (part or all of ppt may be transferred to paper if necessary) and discard filtrate. Place beaker used for pptn under funnel and dissolve ppt on paper in hot 2*N* HCl (20 ml is usually enough if added in several small portions). Wash paper and funnel with 20–30 ml H_2O. Add 2 ml of the 8-hydroxyquinoline soln, 5 ml HOAc (1+9), and quantity of 2*N* NH_4OH equiv. to 2*N* HCl used to dissolve ppt (do not use excess). Dil. to ca 100 ml, heat to 70–90°, and proceed as in (**a**), beginning "slowly add the NH_4OAc soln . . ."

Zinc (*2*)—Official

34.004 REAGENT

8-Hydroxyquinaldine soln.—Dissolve 5.0 g 8-hydroxyquinaldine in 12 ml HOAc, dil. to 100 ml with H_2O, and filter if soln is not clear. (Soln is stable ca one week; if only tech. grade base is available, purify by recrystn from alcohol (2+1), using 6 ml solvent for each g base, before prepg soln.)

34.005 DETERMINATION

Pipet aliquot of sample soln, **34.002,** contg 20–50 mg Zn, into 400 ml beaker. Adjust soln to slight acidity, add 1 g NH_4 tartrate if Al is present, and then add 2 ml of the 8-hydroxyquinaldine soln for each 10 mg Zn present; dil. to 200 ml

and heat to 60–80°. Neutralize excess acid by adding NH_4OH (1+4) until Zn complex salt that forms on addn of each drop just redissolves on stirring. Add slowly, with stirring, 45 ml of the NH_4OAc soln, **34.001(b)**, and let mixt. come to room temp.

Det. pH of soln; if pH is not 5.7–5.9, adjust with the NH_4OH soln, and let mixt. stand 10–20 min. to achieve equilibrium. Decant thru tared gooch and wash ppt in beaker twice with hot H_2O, decanting each washing into crucible. Finally transfer ppt to crucible and again wash with hot H_2O. (Total vol. washings should be >200 ml.) Dry crucible and ppt 2 hr at 130–140°, cool, and weigh. Reheat 30 min. at 130–140°; cool, reweigh, and repeat heating, cooling, and weighing to constant wt. Wt ppt×0.1713 = wt Zn.

34.006 Aluminum—Official

Multiply wt Zn found, **34.005,** by 5.410 to obtain equiv. wt 8-hydroxyquinoline salt, multiply by appropriate factor for aliquot taken, and subtract from wt combined Al and Zn salts, **34.003.** Difference×0.05872 = wt Al.

Boric Acid (*3*)—Official

34.007 PREPARATION OF ION-EXCHANGE COLUMN

Provide glass tube 23″ long×0.75″ diam. with stopcock and outlet tube. Tamp 1″ glass wool plug into bottom of tube, fill tube with H_2O, and add Amberlite IR-120(H) ion-exchange resin slowly to form 8″ column. Wash with HCl (1+9) and then with 50 ml portions H_2O until effluent gives negative Cl test.

Regenerate after use by transferring accumulated resin from number of detns to large glass tube and washing with HCl (1+9) until effluent gives negative test for adsorbed cations, *e.g.*, Zn, Al. Then remove HCl from resin by washing with H_2O until effluent gives negative Cl test.

34.008 DETERMINATION

Place sample contg 50–200 mg H_3BO_3 in 250 ml casserole, add 2 drops phthln, and make alk. with 10% NaOH soln. Evap. to dryness on steam bath under gentle current of air, dry residue 1 hr at 140° in oven, and ash 1 hr at 550°. Cool to room temp., add ca 50 ml hot H_2O, cautiously acidify with HCl, and filter hot soln thru quant. paper into 250 ml beaker. Wash paper with little hot H_2O and reserve filtrate (may be slightly cloudy).

Transfer paper to same casserole and make alk. by wetting with ca 10 ml H_2O and few drops 10% NaOH soln. Evap. to dryness on steam bath, dry 1 hr at 140°, and ash 2 hr at 550°. Cool, add ca 50 ml hot H_2O, acidify with HCl, and filter into reserved filtrate. Wash casserole and paper thoroly with hot H_2O, and discard paper. (Total vol. soln should be ca 200 ml.)

Cool soln; add NH_4OH until barely alk. to litmus paper or until flocculent ppt appears. Reacidify with HCl until slightly acid to litmus paper or until ppt just redissolves. Pass soln thru ion-exchange column into 1 L flask at rate requiring 10–15 min. for passage. Follow sample soln with several 50 ml portions H_2O until effluent is only slightly acid to pH test paper. Add 5 drops methyl red, **2.077(c)**, make alk. with freshly prepd 10% NaOH soln, and then barely acid with HCl.

Connect flask to H_2O-cooled reflux condenser and boil 5 min. Wash down condenser with little H_2O and cool soln to room temp. under running H_2O. Neutralize to methyl red with 0.1*N* NaOH, **42.032**; add 4–5 g mannitol and ca 0.5 ml phthln. Titr. with 0.1*N* NaOH to pink color, add more mannitol, and if pink disappears, continue titrn until it reappears. Repeat addn of mannitol until there is no further change in color.

Det. blank as follows: To ca 350 ml H_2O add quantity of the freshly prepd 10% NaOH soln equal to that required to neutralize sample after passing thru column. Barely acidify with HCl and proceed as above, beginning "Connect flask to H_2O-cooled reflux condenser . . . " Subtract blank titrn from sample titrn and calc. H_3BO_3 content of sample. 1 ml 0.1*N* NaOH = 0.00619 g H_3BO_3.

34.009 Chlorides (*4*)—Official

Pipet aliquot of sample soln, **34.002,** contg ca 100 mg Cl into 250 ml beaker. Dil. to 150 ml with H_2O, neutralize to litmus with NH_4OH (1+1), and acidify with 1 ml HNO_3 (1+1). If any undissolved ppt remains, add more HNO_3 (1+1) until clear soln is obtained. Add dropwise, stirring constantly, slight excess of 0.1*N* $AgNO_3$. (Excess should be not >5 ml.) Pptn and succeeding operations must be carried out in subdued light. Heat mixt. to 90–95° and stir until ppt coagulates. Let ppt settle; add 1–2 drops 0.1*N* $AgNO_3$ to supernatant to assure presence of excess Ag. Let mixt. stand 1–2 hr in dark.

Decant thru tared gooch, wash ppt 2–3 times with 0.01*N* HNO_3 by decantation, and finally transfer ppt to gooch with 0.01*N* HNO_3. Continue washing ppt with 0.01*N* HNO_3 until washing gives negative test for Ag when 1 drop 0.1*N* HCl is added. Complete washing by removing most of the HNO_3 with 1–2 portions H_2O. Dry crucible 2 hr at 120–130° and weigh. Repeat drying to constant wt (0.2 mg). Wt AgCl×0.2474 = wt Cl.

34.010 Sulfates (*4*)—Official

Pipet aliquot of sample soln, **34.002,** contg ca 100 mg sulfate into 600 ml beaker. Dil. to 350 ml

with H_2O, neutralize to litmus with NH_4OH (1+1), and acidify with 2 ml HCl. If any undissolved ppt remains, add more HCl until clear soln is obtained.

Heat 50 ml 1% $BaCl_2$ soln almost to boiling and add rapidly with stirring to the sulfate soln which has also been heated to near b. p. Let ppt settle, and add little $BaCl_2$ soln to assure excess Ba is present. Let mixt. stand 1–2 hr on steam bath. Decant thru tared gooch, wash ppt 4–5 times with small portions of warm H_2O by decantation, and finally transfer ppt to gooch with warm H_2O. Continue washing ppt with warm H_2O until washing gives negative test for Cl. Dry crucible 2 hr at 110–120° and weigh. Repeat drying to constant wt (0.2 mg). Wt $BaSO_4 \times 0.4115$ = wt sulfate.

Methenamine (*5*)—Official

34.011 REAGENT

Borax-carbonate soln.—Dissolve 5.0 g Na_2CO_3 and 4.0 g $Na_2B_4O_7.10H_2O$ in 100 ml H_2O.

34.012 DETERMINATION

Pipet aliquot of sample soln, **34.002,** contg 150–200 mg methenamine into 500 ml round-bottom flask and dil. to 30 ml with H_2O. Neutralize to litmus with either NaOH soln or dil. H_2SO_4; then acidify with 1 ml H_2SO_4. Connect flask to H_2O-cooled condenser and reflux 30 min. to hydrolyze methenamine. Dil. to 175 ml by adding H_2O thru top of condenser, and disconnect condenser. Connect flask thru Kjeldahl trap to efficient straight-wall condenser and distill into 200 ml vol. flask contg 10 ml freshly prepd 10% $NaHSO_3$ soln. Continue distn until residual vol. is ca 5 ml, taking care to avoid charring.

Wash down condenser with little H_2O and cool distillate to room temp. Dil. distillate to mark with H_2O, mix well, and let stand 30 min. Pipet 20 ml aliquot into wide-mouth 250 ml erlenmeyer, add 3–4 ml starch indicator, **4.004(f)**, and destroy excess bisulfite with ca 1*N* I soln. Carefully adjust to starch-I end point with 0.5% $NaHSO_3$ soln and 0.05*N* I. Dil. to 50 ml with H_2O, add 10 ml of the borax-carbonate soln, and titr. with 0.05*N* I to permanent blue. 1 ml 0.05*N* I consumed in alk. titrn = 0.5841 mg methenamine.

Phenolsulfonates

Bromination Method (5)—Official

34.013 REAGENT

Potassium bromate.—0.1*N*. Dissolve 2.8 g $KBrO_3$ in 1L H_2O. Stdze against 0.1*N* $Na_2S_2O_3$.

34.014 DETERMINATION

Pipet aliquot of sample soln, **34.002,** contg 60–125 mg phenolsulfonic acid into 250 ml I flask and dil. to ca 75 ml with H_2O. Add 2–3 ml HCl and 2–3 g KBr, and titr. slowly with 0.1*N* $KBrO_3$ until excess of 1–3 ml is present. (In early stages of titrn, Br formed disappears rapidly but near end point some time is required for Br to react.) Stopper flask and let stand 10 min. If color disappears, add more 0.1*N* $KBrO_3$ and let stand addnl 10 min. Add 2–3 g KI, shake thoroly, and titr. liberated I with 0.1*N* $Na_2S_2O_3$, using starch indicator, **4.004(f)**. I liberated is equiv. to excess $KBrO_3$ soln added. From net vol. $KBrO_3$ soln required in bromination, calc. amount of phenolsulfonic acid in sample. 1 ml 0.1*N* $KBrO_3$ = 0.004355 g phenolsulfonic acid.

Spectrophotometric Method (6)—Official

34.015 APPARATUS

Spectrophotometer.—Capable of isolating wave band of 5 mμ or less in region 220–350 mμ.

34.016 REAGENT

Zinc phenolsulfonate std soln.—10 mg/L in ca 0.1*N* NaOH. Dissolve 100 mg Zn phenolsulfonate, N.F. (equiv. to 62.66 mg phenolsulfonic acid) in 100 ml H_2O. Dil 10 ml aliquot to 100 ml with H_2O. Pipet 10 ml aliquot into 100 ml vol. flask, add 4 ml freshly prepd 10% NaOH soln, and dil. to vol. with H_2O.

34.017 DETERMINATION

(**a**) *In presence of sulfated surface active agents.*—Weigh accurately sample contg 5–10 mg phenolsulfonic acid into 250 ml erlenmeyer. Add 10 ml H_2O and 2 ml HCl, connect to H_2O-cooled condenser, and reflux 0.5 hr. Cool to room temp., transfer to 100 ml separator with aid of 20 ml H_2O, and proceed as in (**b**), beginning "ext. with three 30 ml portions $CHCl_3$."

(**b**) *In absence of sulfated surface active agents.*—Weigh accurately, in weighing bottle, sample contg 5–10 mg phenolsulfonic acid. Transfer to 100 ml separator with aid of 30 ml H_2O. Acidify with HCl and ext. with three 30 ml portions $CHCl_3$. Discard $CHCl_3$ exts. Filter aq. soln thru moistened quant. paper into 100 ml vol. flask and dil. to vol. with H_2O. Pipet 10 ml aliquot into 100 ml vol. flask, neutralize to litmus paper with freshly prepd 10% NaOH soln, add 4 ml excess, and dil. to vol. with H_2O. Det. absorbance of sample, *A*, and std, *A'*, solns at 253 mμ in 1 cm cells, using 0.1*N* NaOH as blank. Calc. % phenolsulfonic acid as follows:

% phenolsulfonic acid = $C \times A/[10A' \times (\text{g sample})]$, where C = concn phenolsulfonic acid (mg/L) in std soln.

34.018 Urea (*4*)—Official

Pipet aliquot of sample soln, **34.002,** contg 50–100 mg urea into 100 ml round-bottom flask with

$\mathbb{T}$ 24/40 female joint. Acidify with HCl, adding 0.5 ml excess. Immerse flask in steam bath and evap. to dryness. Add 10 g cryst. $MgCl_2.6H_2O$ and 1 ml HCl, and connect flask to reflux condenser. Heat mixt. carefully with small flame until the $MgCl_2$ dissolves in its H_2O of crystn, and reflux slowly 2 hr so that rate of return of liquid from condenser is 9–14 drops/min.

Let soln cool, add H_2O thru top of condenser, disconnect flask, and if necessary, heat to dissolve solids. Transfer soln to 1 L flat-bottom flask, dil. to ca 400 ml with H_2O, make alk. with 10% NaOH soln, and distill ca 275–300 ml into suitable portion of $0.1N$ H_2SO_4 contg several drops of methyl red, **42.012(a)**. Titr. excess acid with ca $0.1N$ NaOH, using more indicator if necessary. Stdze the $0.1N$ NaOH against the std $0.1N$ H_2SO_4, using methyl red as indicator.

Correct for blank by refluxing 10 g cryst. $MgCl_2.6H_2O$ and 1 ml HCl and proceeding as above. 1 ml $0.1N$ H_2SO_4 = 3.003 mg urea.

DEPILATORIES

34.019 Sulfides in Powders (*7*)—Official

Pipet 50 ml $0.1N$ As_2O_3 soln, **42.006,** into 250 ml g-s. vol. flask. Weigh sample contg <0.12 g sulfide calcd as H_2S and transfer to flask, washing down with H_2O any material on sides of flask. Add 20 ml HCl (1+1), stopper immediately, and shake vigorously until sample decomposes. (If sample contains $CaCO_3$, add the 20 ml acid slowly thru dropping funnel fitted with rubber stopper to fit flask. Shake gently, letting liberated CO_2 bubble up thru acid. When reaction subsides, drain remainder of acid into flask, remove funnel, stopper flask, and shake vigorously.)

Cool to room temp. and dil. to vol. with H_2O. Filter thru dry paper into dry flask. Pipet 100 ml filtrate into 300 ml erlenmeyer; add 5 ml starch soln, **2.093(d)**, and enough I soln to form blue color. Make alk. with $NaHCO_3$, adding 1–2 g excess. Titr. to permanent blue with $0.1N$ I, **42.016.** Subtract ml $0.1N$ I consumed in alk. titrn from ml $0.1N$ As_2O_3 present in aliquot. 1 ml $0.1N$ As_2O_3 = 0.005411 g CaS or 0.01271 g BaS.

FACE POWDERS (*8*)

34.020 Fats and Fatty Acids as Stearic Acid—Official

Weigh ca 2 g sample into 250 ml g-s. erlenmeyer. Add 30 ml benzene and swirl to mix thoroly. Add 10 ml HCl and swirl, removing stopper frequently to let CO_2 escape from carbonates. When pressure is spent, add 50 ml petr. ether, and shake cautiously, periodically removing stopper until pressure again subsides. Then shake vigorously ca 50 times.

Decant ether layer thru cotton pledget into flask contg few glass beads previously weighed with similar flask as counterpoise. (This decanting involves no danger of loss, for particles of powder are tenaciously retained in acid layer.) Again add 50 ml petr. ether and repeat shaking and decanting. Repeat with third 50 ml portion petr. ether. Evap. solvent soln to dryness on steam bath under hood. Place in force-draft oven 1 hr at 100°, heating flask used as counterpoise at same time. Remove flasks, cool, and weigh as stearic acid.

34.021 Boric Acid—Official

(**a**) *Starch absent.*—Weigh accurately ca 4 g sample and transfer to 500 ml g-s. erlenmeyer. Add 50 ml H_2O, stopper flask, and shake vigorously. With stopper out, heat flask just to boiling, and when cool enough to handle, stopper, shake, and filter thru 12.5 cm medium quant. paper. Transfer residue quantitatively to paper by shaking with small portions of H_2O. Wash residue with H_2O and reserve for detn of Zn. Acidify filtrate to Me orange with $0.5N$ H_2SO_4, adding ca 1 ml excess. Proceed as in **27.014,** beginning "Boil ca 1 min. . . . " ($0.1N$ NaOH may be used in place of the $0.2N$ NaOH specified.)

(**b**) *Starch present.*—Ext. sample with 50 ml cold H_2O by shaking flask vigorously 100 times at 5 min. intervals for 30 min. Filter, and proceed as in (**a**), beginning "Transfer residue quantitatively . . . "

Total Zinc—Official

34.022 REAGENTS

(**a**) *Wulfing precipitant.*—Dissolve 80 g finely ground $(NH_4)_2CO_3$ in mixt. of 90 ml NH_4OH and 375 ml H_2O, and add 475 ml alcohol, which may or may not cause pptn, depending on temp. Let any ppt settle, and use supernatant.

(**b**) *Wash soln.*—Mix equal vols. of the Wulfing precipitant and alcohol.

34.023 DETERMINATION

Weigh ca 2 g sample (or if H_3BO_3 is present, use residue from H_3BO_3 detn, **34.021**) into Pt dish and ignite to light gray ash at 600–650°. Heat no longer than necessary. With aid of wide-mouth funnel, transfer ash to 500 ml g-s. erlenmeyer. Add 100 ml of the Wulfing precipitant so as to wash down funnel. Stopper flask and shake vigorously 1 min., pausing occasionally to remove stopper and relieve pressure. Let stand overnight.

Filter thru 12.5 cm medium quant. paper. Wash out flask with the wash soln, pouring washings thru filter, but make no attempt to transfer residue completely. Reserve flask for later detn of

acid-sol. constituents. Wash residue on paper thoroly with wash soln.

Det. Zn in filtrate as follows: Exactly neutralize to Me red with HCl, add 200 ml H_2O, and bring nearly to boiling on hot plate. Add 60 ml 10% $(NH_4)_2HPO_4$ soln, and continue to heat at just below boiling 30 min. Remove and let cool slowly to room temp. Filter thru weighed gooch previously ignited 10 min. at full heat of Fisher or equiv. burner. Wash with freshly prepd 1% $(NH_4)_2HPO_4$ soln, and finally with 50 ml 50% alcohol. Discard filtrate. Place gooch in porcelain crucible of suitable size, and dry over low flame; then ignite at full heat to constant wt. $Zn_2P_2O_7 \times 0.5341 = ZnO$.

34.024 Acid-Soluble Calcium—Official

Place paper contg residue from Zn sepn in Pt dish and burn off paper at <650°. Transfer ash to 250 ml beaker. Wash residue out of flask used in Zn sepn with 100 ml HCl (1+9), adding washings to beaker. If some residue still clings to inside of flask, wash out with stream of H_2O from wash bottle. Stir thoroly, let stand 10 min., and filter thru medium quant. paper. Disregard turbidity in filtrate. Wash residue on paper 3 times with H_2O. Place in Pt dish not <6 cm diam. nor <2 cm high, and hold pending addn of recovered acid-sol. Fe, Al, and $BaSO_4$.

Nearly neutralize filtrate to Me red with NH_4OH. Add 200 mg $(NH_4)_2SO_4$ and enough Br-H_2O to destroy indicator and distinctly color soln. Boil free of Br, add more Me red, and while still nearly boiling add NH_4OH dropwise to first distinct yellow, avoiding any excess. Let stand ca 3 min., and filter thru medium quant. paper. Wash with hot 2% NH_4Cl soln. Transfer paper and residue to the Pt dish contg acid-insol. constituents. Det. Ca in filtrate as in **4.044,** beginning "heat to boiling . . . "

34.025 Acid-Soluble Magnesium—Official

Det. Mg in filtrate from acid-sol. Ca as in **31.026.** $Mg_2P_2O_7 \times 0.3623 = MgO$.

Barium Sulfate—Official

34.026 APPARATUS

Air bath.—On tripod over Fisher or equiv. burner place 3″ clay triangle holding ca 125 ml Ni or Fe crucible. On top of crucible place 2½″ clay triangle; Pt dish used for fusion rests on this triangle.

34.027 DETERMINATION

Ash residues reserved in Pt dish (acid-insol. portion and materials recovered prior to Ca pptn, **34.024**) at <650°. Pulverize ash with flat-end glass rod, and moisten with 4 ml H_2O. Add 4 ml H_2SO_4, place under hood, and fill dish to ca ¼ its depth with 48% HF. Evap. on air bath, swirling occasionally to mix contents, until only H_2SO_4 appears to remain; then cautiously heat over low flame of Fisher or equiv. burner to pasty consistency, but do not take to complete dryness. Add 15 g pulverized $K_2S_2O_7$, and heat to melting. Continue heating, gradually raising temp. until clear melt is obtained. (Achieved only when dish glows red-hot and melt is orange-red. Too rapid heating will cause spattering. Foaming will occur but is not to be feared. After fusion, clarity of melt may be marred by bubbles and possibly by few flakes of K_2SO_4 produced by the high temp., but these may be disregarded if melt is generally clear.) Set dish aside on asbestos board.

When cool, dislodge melt into 600 ml beaker, wash dish with successive portions of hot H_2SO_4 (1+19) to ca 150 ml, and boil until melt dissolves. $BaSO_4$ ppts at this point; if there is no ppt, dil. soln to 500 ml and proceed as in **34.029.** If there is ppt, digest soln on steam bath 1 hr, let cool, dil. to ca 400 ml, stir well, and let stand at least 2 more hr. Filter thru finest available quant. paper, catching filtrate in 500 ml vol. flask. Wash residue thoroly 3 times with H_2O, transfer to weighed porcelain crucible, burn off paper at low temp., and ignite at dull red heat. Weigh as $BaSO_4$. (Residues amounting to <0.5% should not be counted as $BaSO_4$, as they represent HF-resistant silicate or quartz originally present in talc or kaolin.)

Dil. filtrate from $BaSO_4$ detn to 500 ml and use for detn of Fe, Ti, and Al.

Total Titanium and Iron—Official

34.028 APPARATUS

Jones reductor.—With long glass rod ram pledget of glass wool into the constricted lower end of 50 ml pinchcock buret (without pinchcock attachment). Fill buret to ca 15 ml mark with "20- or 30-mesh" amalgamated Zn. (Zn may be amalgamated by letting it fall into 200 ml H_2O contg 4 g dissolved $HgCl_2$ and 10 ml H_2SO_4. Wash several times with H_2O by decantation before placing in buret.) Fit constricted lower end of buret with 4″ piece of thick-wall rubber tubing with screw-clamp ca midway and terminating in glass tube thrust thru 1 hole No. 7 rubber stopper. Fit stopper to 500 ml vac. flask and adjust glass tube to reach ca 2″ above bottom of flask. When not in use, keep Jones reductor filled with H_2O.

34.029 DETERMINATION

Pipet 100 ml aliquot filtrate from $BaSO_4$ detn, **34.027,** into beaker and add with stirring 5 ml H_2SO_4. Place in vac. flask 10 ml *10% Fe alum*

soln (free of ferrous Fe and other substances reducing $KMnO_4$). Fit flask to reductor, apply vac., and open screw-clamp enough to permit controlled passage of liquid into flask. When meniscus in buret nears level of the Zn, add more soln. (It is preferable never to expose amalgamated Zn to air.) When all soln has been added, add ca 100 ml H_2O in same manner. Close screw-clamp just before meniscus of last washing reaches level of Zn, release vac., and disconnect flask.

Transfer contents to 300 ml tall-form beaker and add 3 ml H_3PO_4. Using 10 ml microburet, titr. over white surface with $0.1N$ $KMnO_4$ to first pink. Prep. blank contg 3 g $K_2S_2O_7$ and 6.5 ml H_2SO_4 in 100 ml H_2O and treat identically as sample, finally titrg to same shade of pink. Subtract titer of blank from that of sample. 1 ml $0.1N$ $KMnO_4 = 0.007988$ g $TiO_2 + Fe_2O_3$ (these have practically same equiv. wt.)

Total Iron—Official

34.030 REAGENT

Titanous chloride std soln.—$0.05N$. Prep. and stdze as in **42.040** and **42.041**, but use only 100 ml 15% $TiCl_3$ soln. Soln may be stdzd in ordinary microburet, titrd in open beaker, kept in g-s. bottle, and restdzd before each set of detns.

34.031 DETERMINATION

Pipet 100 ml aliquot filtrate from $BaSO_4$ detn, **34.027**, into 150 ml beaker. Add 1 g NH_4CNS. Slowly, and with thoro stirring, titr. with the $0.05N$ $TiCl_3$ from microburet to disappearance of red color. Det. blank on 3 g $K_2S_2O_7$ and 6.5 ml H_2SO_4 in 100 ml H_2O. (Blank is often nil.) Correct titer for blank. 1 ml $0.05N$ $TiCl_3 = 0.003993$ g Fe_2O_3.

34.032 Total Titanium—Official

% total $(TiO_2 + Fe_2O_3)$ − % total Fe_2O_3 = % total TiO_2.

34.033 Total Oxides of Iron, Titanium, and Aluminum—Official

Pipet 250 ml aliquot filtrate from $BaSO_4$ detn, **34.027**, into 600 ml beaker. Add few drops of Me red and 5 g NH_4Cl, and bring to boil. Neutralize by adding NH_4OH dropwise to first distinct yellow. Let stand ca 3 min. and filter thru 12.5 cm medium quant. paper. Wash several times with hot 2% NH_4Cl soln. Reserve filtrate for acid-insol. Ca detn, **34.035**.

Place paper in weighed Pt crucible and dry in oven or air bath. Transfer crucible to muffle furnace at room temp., and raise temp. to ca 1100°. Ignite to constant wt. Wt residue is total $Al_2O_3 + Fe_2O_3 + TiO_2$

34.034 Total Aluminum—Official

% total $(Al_2O_3 + Fe_2O_3 + TiO_2)$ − % total $(Fe_2O_3 + TiO_2)$ = % total Al_2O_3.

34.035 Acid-Insoluble Calcium—Official

Det. Ca in filtrate from the NH_4OH ppt, **34.033**, as in **4.044**, beginning "heat to boiling . . ."

34.036 Acid-Insoluble Magnesium—Official

Det. Mg in filtrate from acid-insol. Ca as in **31.026.** $Mg_2P_2O_7 \times 0.3623 = MgO$.

34.037 Silica—Official

Weigh ca 1 g sample into 250 ml beaker. Moisten with alcohol and add 100 ml HCl (1+9). Stir, and let stand 10 min. Filter thru 12.5 cm medium quant. paper. Wash residue 3 times with H_2O. Transfer paper to Pt crucible and ash at <650°. Cool, and pulverize ash with flat-end glass rod. Add 6 g Na_2CO_3, portion at time, intimately mixing with same glass rod between addns. Use last of the Na_2CO_3 to sprinkle over top of mixt. Place in muffle at <800°, and raise temp. to bring contents into fusion. Heat 15 min. at ca 1000°. Remove crucible and let cool.

Dislodge melt into dry 500 ml beaker. (Not always easy; it often helps to return crucible to hot furnace ½ min., then remove and immediately dip ca ⅔ its height in beaker of H_2O. If repeated enough times, this treatment causes melt to crack away from Pt so that it can be removed by simply inverting crucible over beaker.)

Mix 15 ml HNO_3 with 5 ml H_2O in graduate, and wash crucible with successive small portions of mixt., adding washings to beaker. If soln of melt becomes slow, hasten disintegration by gentle pressure with glass rod. When Na_2CO_3 in melt dissolves, place beaker under hood and add, in order named, 5 g NH_4Cl and 25 ml $HClO_4$ (60%). Cover beaker with watch glass and boil over moderate flame until oxides of N pass off and $HClO_4$ refluxes down sides of beaker. Cool mixt. slightly, add 150 ml very hot H_2O, stir, and let stand until SiO_2 settles.

Decant thru 12.5 cm medium quant. paper, and transfer residue to paper, using hot H_2O and policing beaker. Wash thoroly 5 times with hot H_2O. Transfer to Pt dish, burn off paper, and ignite to constant wt at ca 1100°. Weigh as crude SiO_2.

To residue in dish add ca 2 ml H_2SO_4 (1+9) and enough 48% HF to cover the SiO_2. Heat on steam bath under hood until SiO_2 and excess HF have evapd. Cautiously heat over non-reducing flame of Fisher or equiv. burner until fumes of SO_3 cease to be evolved, and then heat strongly

several min. Cool and reweigh. Difference between wt crude SiO_2 and this wt = wt SiO_2.

34.038 **Starch—Official**

Weigh ca 5 g sample into 500 ml Florence flask (preferably ₸). Moisten with 10 ml alcohol. Acid-wash as in **7.018**, hydrolyze starch as in **22.043** (but filter hydrolyzed mixt. before and not after dilg to vol.), and det. dextrose as in **29.039** and **29.040**.

HAIR PREPARATIONS

2,5-Diaminotoluene in Hair Dyes and Rinses (*9*)—First Action

34.039 *Acetylation Method*

Place in small separator 5–20 ml aliquot sample soln contg 0.05–0.15 g of the diamine. Add ca 0.05 g Na_2SO_3 and vol. NaOH soln (1+1), **42.031(b)**, equal to 55% of previously measured vol. of sample soln. (It is convenient to add the NaOH soln from wide-tip Mohr pipet.) Cool contents of separator as rapidly as possible, add 20 ml ether, and shake gently ca 30 sec. Let stand ca 1 min. and drain NaOH layer into second separator, taking care to remove all aq. layer. (It does no harm to transfer 1–2 ml of ether layer also.)

Carefully decant ether layer thru cotton pledget in long-stem funnel into tared evapg dish in such way that none of aq. layer that drains from sides of funnel is transferred with the ether. Wash first separator with 20 ml ether and drain ether into separator contg alk. layer. Make second extn as before, return aq. layer to first separator, and decant ether layer thru cotton pledget into evapg dish. Continue in this manner until 5 extns are made.

Wash funnel and cotton with little ether and evap. combined exts on steam bath to ca 10 ml. Add 1 ml Ac_2O (<0.003% non-volatile material) and continue evapn to dryness. Add few ml alcohol and evap. until Ac_2O odor disappears. Dry residue 15 min. at 100°, cool, and weigh diacetyl derivative of the diamine. Repeat drying to constant wt (±0.5 mg). Diacetyl 2,5-diaminotoluene ×0.5924 = 2,5-diaminotoluene.

Check purity of diacetyl derivative by detg its m.p.; m.p. diacetyl 2,5-diaminotoluene is 219–220°.

Dichlorimide Method

34.040 REAGENTS

(a) *Sodium hypochlorite soln.*—5% U.S.P. soln of NaOCl.

(b) *Sodium arsenite soln.*—10%. Dissolve 10 g $NaAsO_2$ in 100 ml H_2O; or dissolve by heating 8.5 g As_2O_3 and 15 g NaOH in 100 ml H_2O.

34.041 DETERMINATION

To separator contg 5 ml of the NaOCl soln and ca 1 g $NaHCO_3$, add, from pipet or buret, aliquot of sample soln contg 0.01–0.08 g diamine. If insufficient NaOCl is indicated by presence of brown color while soln is being added, repeat operation, using more NaOCl or smaller aliquot. Thoroly mix soln during addn by gently swirling separator. Stopper separator and shake ca 10 sec. Add 10 ml of the $NaAsO_2$ soln, stopper separator, and shake again.

Ext. dichlorimide with two 25 ml portions $CHCl_3$ and combine exts in second separator. Wash combined exts with 10 ml H_2O and filter thru cotton pledget into I flask. Make addnl extn, wash with the H_2O, and combine with major portion. Add 50 ml H_2O contg 1 g KI and 3 ml HCl to combined $CHCl_3$ exts, stopper flask, and shake vigorously 1 min. Titr. liberated I with 0.1N $Na_2S_2O_3$. Stopper flask and shake vigorously at intervals during titrn. (The I in the $CHCl_3$ acts as indicator.) Toward end of titrn add starch soln, **2.093(d)**, for final end point. 1 ml 0.1N $Na_2S_2O_3$ = 0.002036 g 2,5-diaminotoluene.

Paraphenylenediamine in Hair Dyes and Rinses—Official

Acetylation Method (10)

34.042 PREPARATION OF SAMPLE

(a) *Powders or dry mixtures.*—Place 1–2 g of the powder directly into 50 ml vol. flask, add 2 ml HCl (1+1), and dil. to vol. with H_2O.

(b) *Aqueous preparations.*—Dil. if necessary so that 5 ml aliquot contains 0.1–0.3 g p-phenylenediamine.

34.043 DETERMINATION

Pipet 5 ml aliquot prepd soln into continuous extractor such as that shown in Fig. 63, page 475, and add enough anhyd. Na_2CO_3 to render aq. layer alk. to litmus paper. Completely ext. with $CHCl_3$, remove flask, and transfer $CHCl_3$ soln to 100 ml beaker, rinsing flask with few small portions of $CHCl_3$. Evap. $CHCl_3$ to ca 25 ml and add slowly, with stirring, 1 ml Ac_2O. Let stand 1 hr and filter on weighed gooch. Wash beaker and ppt with three or four 5 ml portions $CHCl_3$. Use great care in removing last traces of ppt from beaker. Dry to constant wt at 120° and weigh ppt of diacetyl p-phenylenediamine, $C_6H_4(NHCOCH_3)_2$, m.p. 312–314°. Diacetyl derivative ×0.5626 = p-phenylenediamine.

Check purity of diacetyl derivative by detn of m.p.

34.044 *Dichlorimide Method (Benzoquinone Method)* (*11*)

Proceed as in **34.041.** 1 ml 0.1*N* $Na_2S_2O_3$ =0.001802 g *p*-phenylenediamine.

Potassium Bromate and Sodium Perborate in Cold Wave Neutralizers—Official

34.045 *Qualitative Tests* (*12*)

(**a**) *General tests.*—$KBrO_3$ and $NaBO_3$ are white cryst. salts sol. in H_2O. Aq. soln of $KBrO_3$ is slightly acid; of $NaBO_3$, slightly alk. In flame test, using Pt wire in slightly darkened room, $KBrO_3$ gives reddish-violet flame when viewed thru Co glass; $NaBO_3$, typical yellow Na flame. Both compounds give following test: Dissolve 0.1 g sample in 10 ml H_2O, acidify with HCl, and add 0.5 g KI. Liberation of I indicates presence of oxidizing agent.

(**b**) *Confirmatory test for bromate.*—To 1 ml 5% soln of sample in test tube add slowly with vigorous shaking 2 ml H_2SO_4. Note odor and color of liberated gas. (*Caution.*) Cool test tube, *carefully* add 2 ml CS_2, and shake. CS_2 layer becomes yellow or red if Br is present.

(**c**) *Confirmatory test for boron.*—Moisten 0.2 g sample in porcelain crucible with 1–2 drops H_2SO_4, add 2 ml MeOH, stir well, and ignite. Green flame indicates presence of B.

Pyrogallol in Hair Dyes (*13*)—Official

34.046 *Qualitative Test*

Add 5–10 ml sample to separator contg ca 0.5 g $NaHSO_3$ and ext. with two or three 30 ml portions ether. Filter ether exts thru cotton and evap. to dryness on steam bath. Dry 30–60 min. at 100°. Pulverize residue, mix well, and take m.p. If residue does not melt at 131–134°, sublime and again take m.p., which should fall within this range. Mix small portion of residue with equal quantity of sublimed pyrogallol and det. m. p., which should not change.

Quantitative Determination

34.047 REAGENTS

(**a**) *Ferrous tartrate reagent.*—Dissolve 1.00 g Na K tartrate (Rochelle salt) and 0.200 g $FeSO_4.7H_2O$ in H_2O, and dil. to 100 ml in vol. flask. Prep. fresh daily.

(**b**) *Sodium acetate soln.*—Dissolve 15.00 g $NaOAc.3H_2O$ in H_2O, bring to room temp., and dil. to 100 ml.

(**c**) *Pyrogallol std soln.*—0.2000 g/L.

34.048 PREPARATION OF STANDARD CURVE

To six 100 ml vol. flasks add, from buret, 2.50, 5.00, 7.50, 10.00, 12.50, and 15.00 ml of the std pyrogallol soln. Develop color as follows on not >3 stds at time, and make readings within 15 min. after color is developed:

Pipet into flasks 10 ml each of the NaOAc soln and Fe tartrate reagent, dil. to vol., and mix. Using 1 cm or ½″ cells, measure absorbance of solns with photometer or spectrophotometer at 540 mμ. (With neutral wedge filter photometer, filter designated No. 56 (5.8 mm Corning didymium No. 512, 2.0 mm Jena VG 3, 2.0 mm Jena BG 18, and 4.5 mm Corning yellow shade yellow No. 351) is more suitable than filter No. 54.)

With filter photometers obtain zero point by reading blank soln contg 10 ml each of the NaOAc soln and Fe tartrate reagent in 100 ml. Draw std curve, plotting concns pyrogallol against photometer readings, on large-scale graph paper so that concn can be read to 0.01 mg. Straight line should be obtained between concns of 1–6 mg/100 ml. With spectrophotometer use freshly prepd blank as reference soln. Draw std curve as above, or, if straight line passing thru origin is obtained, av. value of absorptivity, a, may be calcd from formula: $a = A/bc$, where A is measured absorbance; b is cell length; and c is concn pyrogallol. This value of a may be used to calc. concn of unknowns directly from absorbance.

34.049 DETERMINATION

(**a**) *Liquid dyes.*—Ext. convenient aliquot of sample (usually 10 ml) by one of following methods with min. exposure to air, as pyrogallol is readily oxidized:

(**1**) *Continuous extraction.*—Pipet sample aliquot into suitable continuous extractor contg ca 0.3 g $NaHSO_3$. Ext. with ether until pyrogallol is completely removed (3–7 hr, depending upon efficiency of extractor). (Det. time required for each extractor by extg aq. soln of known pyrogallol content or by testing for complete extn as follows: After extn is considered complete, remove flask contg ether, replace with one contg fresh ether, and continue extn 30–60 min. Treat this ext. as below, and use 50 ml aliquot filtrate to develop color.) Evap. ether ext. on steam bath to 8–10 ml and continue evapn at temp. not >40° until odor of ether is completely gone.

Dissolve residue in 20 ml H_2O and wash completely into 100 ml vol. flask. Dil. to vol. and mix. (If liquid sample contains chlorophyll, treat residue from ether extn with alumina cream as in (**b**), beginning "Add 10 ml H_2O and loosen residue . . . ") Filter thru dry paper and discard first 20 ml filtrate. (If detn cannot be completed same day extn is made, let ether ext. stand overnight, preferably in refrigerator, before ether is evapd. Do not let aq. soln stand overnight.) Use

suitable aliquots of filtrate to develop color as in **34.048**, beginning "Pipet into flasks . . . " If 5 ml aliquot contains >6 mg pyrogallol, make suitable diln in vol. flask and use aliquots of dild soln to develop color. For final calcn use av. of results obtained on at least 2 aliquots of different sizes, preferably contg 2–5.5 mg pyrogallol. Calc. to g/100 ml in original sample.

(2) *Extraction in separators.*—Pipet sample into 125 ml separator contg ca 0.3 g $NaHSO_3$ and ext. 6 times with ether. For each extn use vol. ether equal to 3 or 4 times vol. sample and shake vigorously 1 min. Filter ether exts successively thru cotton wet with ether. (6 extns carefully made will completely remove pyrogallol, but seventh may be made and used to test for complete extn as in (1).) Evap. combined ether exts and proceed as in (1).

(**b**) *Henna powder mixture.*—Weigh 0.9–1.1 g thoroly mixed sample into paper extn thimble. Cover sample with small piece of cotton and place thimble in Soxhlet app. If temp. and humidity are such that H_2O will condense on condenser, connect tube contg drying agent to outlet of condenser. Ext. 5 hr with EtOAc, 99% min. purity. Boil at such rate that solvent siphons off 15–20 times/hr.

If EtOAc ext. is clear, evap. to dryness as below. If ext. contains any sediment, evap. to ca 75 ml if necessary, cool to room temp., and completely transfer to 110 ml g-s. vol. flask. Dil. to vol. and mix. Filter thru dry paper, taking precautions to prevent evapn of solvent. Pipet 100 ml filtrate into 250 ml beaker and evap. to ca 5 ml on hot plate or steam bath. Continue evapn to complete dryness at temp. not >40°. Add 10 ml H_2O and loosen residue with stirring rod. Pour into 50 ml vol. flask. Rinse beaker 4 or 5 times with small vols H_2O and add rinsings to flask. Add 1.2 ml alumina cream, **29.021(b)**, dil. to vol., mix, and filter thru dry paper. Ext. 25 ml filtrate by one of methods given in (**a**). If extn cannot be started immediately, add ca 0.4 g $NaHSO_3$ to filtrate and hold no longer than overnight. Calc. to % pyrogallol in original sample.

Resorcinol in Hair Lotions (*14*)—Official

34.050 REAGENTS

(**a**) *Potassium iodide soln.*—Dissolve 25 g KI in H_2O and dil. to 100 ml.

(**b**) *Sodium thiosulfate std soln.*—0.1*N*. Stdze as in **42.036**.

(**c**) *Bromide-bromate std soln.*—0.1*N*. Dissolve 3 g $KBrO_3$ and 15 g KBr in H_2O and dil. to 1 L. Pipet 25 ml of this soln into 500 ml I flask and dil. with 100 ml H_2O. Add 5 ml HCl, stopper flask, and shake gently. Add 5 ml of the KI soln and shake mixt. Titr. with the $Na_2S_2O_3$ soln, using starch indicator, **2.093(d)**.

34.051 DETERMINATION

Pipet 25 ml sample into 150 ml beaker. Dealcoholize as follows: Place beaker on covered surface of steam bath and direct air current from fan over beaker. Evap. to ca 10 ml, add 15 ml H_2O, and again evap. to 10 ml. Transfer contents to separator with several small portions of H_2O to final vol. of 30 ml. Complete transfer by washing beaker with several portions of $CHCl_3$, totaling 25 ml. (Most of solid material remaining after dealcoholization is transferred by the $CHCl_3$ rather than by the H_2O.) Acidify with 1–2 ml HCl (1+9).

Ext. with two addnl 25 ml portions $CHCl_3$. Wash each ext. with same 5 ml portion H_2O. Discard $CHCl_3$, and add wash H_2O to residual acid aq. layer. Ext. aq. layer with five 35 ml portions ether. Add 10 ml H_2O to combined ether exts. Evap. the ether, at low temp., on surface of steam bath in air current from fan. Transfer aq. soln to 100 ml vol. flask, cool, dil. to vol., and mix.

Transfer portion of this soln (aliquot taken should require 20–40 ml 0.1*N* KBr-$KBrO_3$) to I flask. Add 50 ml of the KBr-$KBrO_3$ soln, dil. with 50 ml H_2O, and add 5 ml HCl. Immediately stopper and shake flask, and let stand 1 min. Remove stopper just enough to add 5 ml of the KI soln, taking care that no Br vapors escape. Immediately stopper and shake flask thoroly, remove stopper, and rinse it and neck of flask with 20 ml H_2O. Titr., *at once*, with the $Na_2S_2O_3$ soln, using starch indicator, **2.093(d)**. 1 ml 0.1*N* KBr-$KBrO_3$ = 0.001835 g resorcinol.

34.052 Salicylic Acid in Hair Lotions (*15*)—Official

Acidify 25 ml sample in 250 ml beaker with 2 ml HCl (1+3). Dealcoholize by heating at not >70°; if possible, by use of air current at room temp. Transfer to separator and dil. with H_2O to ca 25 ml. Since $CHCl_3$ is to be used later as extg solvent, make transfer by washing from beaker to separator with 2 or 3 portions of $CHCl_3$, totaling 25 ml, repeating washing with H_2O in portions totaling 25 ml, thus obtaining indicated aq. diln.

Ext. with four 25 ml portions $CHCl_3$ (including 25 ml used in transfer of sample to separator). Wash each $CHCl_3$ ext. with same 5 ml H_2O and filter into 150 ml beaker thru $CHCl_3$-satd cotton pledget. Wash the 5 ml H_2O with $CHCl_3$, filtering this $CHCl_3$ washing into same beaker. Evap. $CHCl_3$ on steam bath to 20–25 ml, and then evap. spontaneously to 5 ml.

Transfer remaining 5 ml to separator with enough $CHCl_3$ as rinse to total ca 30 ml in separator. Ext. with three 5 ml portions 5% $NaHCO_3$

soln and one 5 ml portion H_2O. Wash combined aq. exts with 10 ml $CHCl_3$ and discard the $CHCl_3$.

Filter combined $NaHCO_3$ solns thru paper into 100 ml vol. flask. Rinse separator and wash filter with H_2O until filtrate reaches 100 ml mark, and mix.

Proceed as in **32.129(b)**, line 4, beginning "Transfer aliquot of this soln . . . " Before adding the 0.1*N* KBr-$KBrO_3$, carefully neutralize aliquot in I flask to liberate CO_2 from bicarbonate present; then make alk. with 1 drop 10% NaOH soln and continue as directed. 1 ml 0.1*N* KBr-$KBrO_3$ = 0.002302 g salicylic acid.

Thioglycollate Solutions in Cold Permament Waves *(12)*—Official

34.053 *Qualitative Test*

Dil. 2 ml sample to 10 ml with H_2O, acidify with 10% HOAc, add 5 ml excess, and shake well. Add 2 ml 10% $Cd(OAc)_2.2H_2O$ soln, and shake. White gelatinous ppt forms if thioglycollic acid is present. Add excess of NH_4OH (2+3) and shake. Ppt of Cd thioglycollate will dissolve.

34.054 *Quantitative Method*

(Applicable in absence of reducing substances other than thioglycollates)

Pipet sample aliquot contg 250–300 mg thioglycollic acid into wide-mouth 250 ml erlenmeyer. Dil. to 50 ml with H_2O, add 2–3 drops Me red, **2.077(c)**, and make slightly acid with HCl. Add 3–4 ml starch indicator, **4.004(f)**, and titr. with 0.1*N* I to purple end point. 1 ml 0.1*N* I = 0.009212 g thioglycollic acid.

VANISHING CREAM *(16)*

34.055 Test for Type of Emulsion—Official

Dust small quantities of finely ground oil-sol. and H_2O-sol. dyes on sep. portions of sample. If color of oil-sol. dye spreads rapidly, H_2O-in-oil emulsion is indicated; if color of H_2O-sol. dye spreads, oil-in-H_2O emulsion is indicated.

34.056 Water—Official

Transfer 5–20 g sample to erlenmeyer; add 50 ml toluene, few glass beads, and ca 2 g *lump rosin*. Connect flask to Dean and Stark distg tube receiver, and distill until no more H_2O collects in receiver. Cool, read vol. H_2O under the toluene at room temp., and from this vol. calc. % H_2O.

34.057 Ash—Official

Place 2–10 g sample in flat-bottom Pt dish, and remove H_2O and volatile material by placing dish on steam bath or in 100° oven. Ignite sample at low temp. and finally at 600° to constant wt.

34.058 Chloroform-Soluble Material—Official

Place 2–10 g sample in separator, add 25–50 ml H_2O, acidify slightly with H_2SO_4 (1+9), and ext. with successive portions of $CHCl_3$, collecting all exts in second separator. (Usually 4–5 portions of $CHCl_3$, each ca 35 ml, are enough to remove all $CHCl_3$-sol. material.) Wash combined $CHCl_3$ exts with 10 ml H_2O, filter thru cotton plug placed in separator stem, and collect filtrate in weighed dish. Shake aq. washing with small quantity of $CHCl_3$, and filter this $CHCl_3$ into dish. Evap. $CHCl_3$ on steam bath and dry residue for 15 min. intervals at 100° to constant wt.

Glycerol—Official

34.059 REAGENTS

(**a**) *Potassium periodate soln.*—0.02*M*. Dissolve 4.6 g KIO_4 in ca 500 ml hot H_2O. Dil. to ca 900 ml with H_2O, cool to room temp., and dil. to 1 L.

(**b**) *Sodium hydroxide std soln.*—0.02*N*. *See* **42.033** or **42.034.**

(**c**) *Bromocresol purple indicator.*—Dissolve 0.1 g bromocresol purple in 100 ml alcohol.

(**d**) *Propylene glycol.*—B.p. 85–86°/10 mm.

(**e**) *Arsenious oxide soln.*—0.02*N*. Dil. 100 ml 0.1*N* As_2O_3, **42.006,** to 500 ml with H_2O.

34.060 ISOLATION AND OXIDATION OF GLYCEROL

(**a**) *Isolation of glycerol.*—Place 2–10 g sample in separator, add 25–50 ml H_2O, acidify slightly with H_2SO_4 (10 g/100 ml), and ext. with successive portions of $CHCl_3$. (Usually 4–5 portions, each ca 35 ml, remove all $CHCl_3$-sol. material.) Wash combined $CHCl_3$ exts with 10 ml H_2O. Filter aq. soln and wash H_2O thru cotton plug to remove droplets of $CHCl_3$, and collect filtrate in 250 ml vol. flask. Add 3 drops of the bromocresol purple indicator to filtrate and neutralize with CO_2-free alkali (0.1*N* NaOH is satisfactory), making final adjustment with the 0.02*N* NaOH. Dil. almost to mark with H_2O, and if necessary add more alkali to maintain light but definite purple color in soln; then complete diln to mark and mix.

(**b**) *Periodate oxidation.*—Transfer aliquot of the neutral soln, preferably contg 30–40 mg glycerol, to 100 ml vol. flask, and add 50 ml of the KIO_4 soln. Dil. to mark with H_2O and let stand ca 1 hr. Test for excess periodate, which must be present in oxidation mixt., by adding $NaHCO_3$ and KI to test portion. If excess is present, I is liberated.

34.061 DETERMINATION

(**a**) *By titration of formic acid.* (Applicable in absence of substances yielding acid on periodate

oxidation.) Transfer 50 ml aliquot of oxidized mixt. to titrn flask, add 10 drops of the propylene glycol (ca 0.5 ml), mix well, wash down sides of flask with H_2O, and let stand 10 min. Add 3 drops of the bromocresol purple indicator and titr. with the NaOH soln to light purple end point. 1 ml 0.02*N* NaOH = 1.842 mg glycerol.

(b) *From periodate consumed.*—Transfer 20 ml aliquot of oxidized mixt., **34.060**(b), to titrn flask and dil. with ca 50 ml H_2O. Add ca 1.0 g $NaHCO_3$, 0.5 g KI, and 5 ml starch indicator, **2.093**(d). Titr. immediately with the As_2O_3 soln to disappearance of blue color. Stdze 10 ml of the KIO_4 by same titrn procedure. Difference between the 2 titrns represents quantity of periodate reduced in 20 ml aliquot taken. To obtain quantity of periodate reduced in original aliquot obtained from 250 ml flask, multiply above difference by 5. 1 ml 0.02*N* As_2O_3 = 0.4605 mg glycerol.

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(*4*) J. Assoc. Offic. Agr. Chemists **34**, 299 (1951).

(*5*) Ibid. **35**, 279(1952).

(*6*) Ibid. **37**, 798(1954).

(*7*) Ibid. **23**, 440(1940); **25**, 113(1942); **27**, 112 (1944).

(*8*) Ibid. **25**, 909(1942); **32**, 50, 601(1949); **33**, 359(1950).

(*9*) Ibid. **22**, 159(1939); **26**, 116(1943); **33**, 374(1950).

(*10*) Ibid. **23**, 717(1940); **33**, 374(1950).

(*11*) Ibid. **22**, 159(1939); **25**, 113(1942); **27**, 112(1944).

(*12*) Ibid. **35**, 285(1952).

(*13*) Analyst **48**, 2(1923); **50**, 49(1925); J. Assoc. Offic. Agr. Chemists **28**, 744(1945); **30**, 512(1947); **31**, 577(1948); **32**, 592(1949).

(*14*) J. Assoc. Offic. Agr. Chemists **25**, 897 (1942); **30**, 517(1947).

(*15*) Ibid. **25**, 112(1942); **26**, 355(1943); **27**, 112(1944).

(*16*) Ibid. **25**, 903(1942); **26**, 249(1943); **27**, 462(1944); **30**, 507, 651(1947); **31**, 580(1948); **33**, 362, 367(1950).

35. Coloring Matters

(Number in brackets following name of a dye represents number of that dye as listed in Society of Dyers and Colourists' "Colour Index," second edition, 1956.

In conformity with common usage, thruout this chapter the reagent designated as "amyl alcohol" is actually "*iso*-amyl alcohol.")

SEPARATION AND IDENTIFICATION OF COLORING MATTERS IN FOODS, DRUGS, AND COSMETICS

35.001 *Pigments and Lakes—Procedure*

Sep. insol. pigments, ultramarine, lampblack, etc., that are most commonly used as facings, by washing sample with H_2O and letting washings settle. Identify particles of coloring matter by microscopic examination and treat residue or purified coloring matter with chemical reagents.

Pigments occasionally encountered are charcoal or other form of C, ultramarine blue (principally Al, S), Prussian blue (principally Fe), and talcum (principally SiO_2). Charcoal is indifferent towards usual chemical reagents and can be burned. Ultramarine blue is stable towards alkalies, but is decomposed by dil. HCl with evolution of H_2S. Prussian blue is unaffected by dil. HCl, but is decomposed by alkalies. Talcum can be confirmed by purple color obtained by fusing with $Co(NO_3)_2$ (test for Al).

Lakes are products formed by combining org. coloring matters with metallic salts. They can be prepd from animal or vegetable coloring matters or from coal-tar dyes. As a rule they are insol. in H_2O, but are readily decomposed by acids with liberation of the coloring matter.

Large proportions of common pigments other than lakes, such as yellow, brown, and red ochres and umbers, are derivatives of heavy metals and contain Fe, Mn, etc. Others, such as green and blue compounds, including certain green chlorophyll derivatives, may contain Cu. These pigments may be identified by usual tests for respective metals. Analytical properties of insol. coloring matters are described in various std works, some of which are listed in the selected references, especially *Farbstofftabellen* by Schultz (*1*) and *Colour Index*.

Soluble Coloring Matters and Their Lakes

Separation by Immiscible Solvents (2)

35.002 Coal-Tar Dyes in General—Official

Use of immiscible solvents to sep. mixtures of coloring matters generally requires systematic fractionation, since many dyes do not differ very greatly in their solubilities in various solvents. These sepns may also be accomplished by column and paper chromatography.

35.003 Oil-Soluble Coal-Tar Dyes (*3*)—First Action

Ext. oil or fat with petr. ether and treat petr. ether ext. as follows:

(**a**) Ext. with 10–25 ml portions HCl-HOAc mixt. (0.5 part H_2O, 1 part HCl, 5 parts HOAc) until colorless or nearly so. Make acid exts alk. with ca 25% NaOH soln and re-ext. with petr. ether of low b.p. Wash petr. ether ext. free of excess alkali, and evap. Take up residue in 70% alcohol and identify. Dyes present may be aniline yellow, butter yellow, aminoazotoluene, sudan G or sudan I, yellow AB or OB, orange SS, or oil red XO.

(**b**) If petr. ether soln is not colorless after extn with the HCl-HOAc mixt., ext. with 5–15 ml portions H_2SO_4-HOAc mixt. (1 part H_2O, 4 parts H_2SO_4, 9 parts HOAc) until colorless. Make combined H_2SO_4-HOAc exts. alk. with the NaOH soln, re-ext. with petr. ether, evap. petr. ether ext., and take up residue in 70% alcohol as in (**a**). Dyes present may be sudan III or IV, oil red OS, or quinizarine green SS.

In all cases test 2 small portions of alc. soln by mixing one with equal vol. HCl and other with equal vol. 40% $SnCl_2$ soln. Common oil-sol. dyes are made redder or bluer with acid and are decolorized by $SnCl_2$. Most natural coloring matters become slightly paler with acid and are little changed by $SnCl_2$.

Water-Soluble Coal-Tar Dyes (*2*)—Official

35.004 PREPARATION OF SOLUTION

(**a**) *Water-soluble colors.*—Obtain aq. soln as free as practicable from suspended matter, alcohol, acids, alkalies, and salts. Liquids require no prepn except removal of alcohol.

(**b**) *Water-insoluble lakes.*—If sample is in solid form, treat well-divided material with enough H_2O to form paste.

35.005 Basic Dyes

Most basic dyes may be sepd from mixts by making prepd soln, **35.004**, alk. with 10% NaOH

soln and shaking with ether (4). Sep. ether layer, which may or may not be colored; wash twice with few ml H_2O to remove excess of alkali; and shake with HOAc (1+18), which takes up any dye present and forms colored soln. Altho this treatment may, to some extent, alter common basic colors, it can be used to detect Me violet B [42535], magenta [42510], bismarck brown [21000], malachite green [42000], and rhodamine B [45170]. With care auramine [41000] also may be sepd in this way, tho it quickly decomposes on standing in alk. soln.

35.006 Acid Dyes

Following short procedure is often convenient for examination of mixts of acid dyes: Make prepd sample, **35.004**, strongly acid by adding ½ its vol. of HCl, and shake with amyl alcohol. Sep. amyl alcohol soln and wash by shaking with successive portions of ½ its vol. of H_2O, reserving portions in sep. test tubes or beakers. Because of varying acid content of the amyl alcohol these washings will show regular decrease in acidity, and coloring matters will appear in max. quantity in different fractions according to their respective solubilities.

Ponceau 6R [16290] is washed out chiefly while acidity is still high, ca 1*N*. Amaranth [16185], brilliant scarlet [16255], tartrazine [19140], sunset yellow FCF [15985], orange G [16230], and sol. blue [42755] appear when washings have acidity of ca 0.25*N*, and palatine scarlet [14900], ponceau 2R [16150] and 3R [16155], ponceau SX [14700], naphthol yellow S [10316], cochineal [75470], crystal ponceau [16250], and azorubine A [14720], between 1/16*N* and 1/256*N*.

When practically all acid is removed, orange I [14600], orange II [15510], and croceine orange [15970] begin to wash out, and, less readily, orange IV [13080] and metanil yellow [13065). Finally unsulfonated coloring matters, such as erythrosine G [45425], erythrosine B [45430], and the rose bengals [45435] and [45440] are removed very slowly by H_2O or not at all unless solvent is dild with petr. ether and dyes are removed with H_2O contg few drops of NH_4OH.

Acid yellow [13015] and brilliant yellow S [13085] are not uniform in composition. They are partially taken up by amyl alcohol from acid soln and appear chiefly in first washings. Indigotine [73015] behaves somewhat similarly.

When it appears probable that only coal-tar dyes presently or previously listed in regulations for enforcement of Federal Food, Drug, and Cosmetic Act (5) for use in food products are present, following abridged procedure may be conveniently used for their sepn:

Permitted Coal-Tar Food Colors (6)

(Amaranth, ponceau 3R, ponceau SX, erythrosine, orange I*, light green SF yellowish, fast green FCF, guinea green B, brilliant blue FCF, indigotine, naphthol yellow S**, sunset yellow FCF, tartrazine, yellow AB**, yellow OB**, orange SS*, and oil red XO*.)

35.007 Preparation of Solution—First Action

(**a**) *For foods containing oil-soluble dyes.*—Shake oil or melted fat with equal vol. alcohol, 90% by vol., wash alc. ext. with several portions of petr. ether to free coloring matter from fats, and then evap. alc. ext. to dryness in casserole. Treat residue with 40 ml petr. ether, and shake petr. ether soln with two or three 5 ml portions 2–4% NaOH soln (to remove annatto, turmeric, etc., if present). Petr. ether soln will contain yellow OB, yellow AB, orange SS, and oil red XO.

(**b**) *For foods containing no oil-soluble dyes or from which these dyes have been removed.*—Obtain aq. soln as free as possible from suspended matter, alcohol, acids, alkalies, and salts. Dye soln should preferably be 0.01–0.05%. Soln obtained in examination of colored food products rarely requires further diln, but with commercial food colors care must be taken that concn is not too great.

35.008 Separation—Official

(a) *Yellow AB and yellow OB.*—Ext. petr. ether soln of these dyes, **35.007**(**a**), 3 times with ½ its vol. 13*N* H_2SO_4. Shake each acid ext. successively with 2 portions (equal vols) of petr. ether, using same 2 portions of petr. ether for each acid portion. Ext. each of 2 latter petr. ether portions with 20 ml 13*N* H_2SO_4, using same acid portion successively for both petr. ether portions. Finally ext. second of these petr. ether portions with another 20 ml portion 13*N* H_2SO_4. (Original petr. ether soln has now been shaken with acid 3 times, next petr. ether portion 4 times, and third 5 times.) Combine acid exts, dil. with H_2O, re-ext. with petr. ether, and evap. solvent. Yellow AB will be found in practically pure state.

Combine petr. ether solns (original and subsequent solns left after acid washings), wash with small portions of H_2O to remove excess of acid, and evap. solvent. Yellow OB will remain as residue. (This method is not absolutely quant., but it is accurate enough to sep. either dye with comparatively little contamination from other.) Following color tests may be applied to sepd dyes to confirm identity:

(1) Shake 5 ml neutral petr. ether soln of dye in test tube with 5 ml mixt. of 1 part 40% HCHO

* Not permitted in United States as of Feb. 16, 1956.
** Not permitted in United States as of May 6, 1959.

soln and 4 parts Ac_2O. Both coloring matters are extd by Ac_2O, yellow AB giving red soln in few sec., and yellow OB, under same conditions, giving orange soln.

(2) To 1 ml alc. soln of dye (0.005–0.01%), add 0.1 ml HCHO, 0.1 ml H_2SO_4, and finally 8.0 ml H_2O. Yellow AB gives red color, unaltered by addn of excess NH_4OH and somewhat intensified by further addn of excess HOAc. Yellow OB gives yellow or orange color.

(3) To 1 ml alc. soln of dye (0.005–0.01%), add 0.1 ml *Cu-pyridine soln* (5 g $CuSO_4.5H_2O$ and 10 ml pyridine dild to 100 ml with H_2O) and 8.0 ml H_2O. Yellow AB gives pink color, becoming purple on addn of excess NH_4OH. Yellow OB gives colorless or bluish soln.

(**b**) *Amaranth, ponceau 3R, ponceau SX, erythrosine, orange I, light green SF yellowish, fast green FCF, guinea green B, brilliant blue FCF, indigotine, naphthol yellow S, sunset yellow FCF, and tartrazine.*—To soln obtained in **35.007(b)** add enough 25% NaCl soln to make concn ca 10%, and 1 part HOAc to every 7 parts soln. Ext. with three 50 ml portions amyl alcohol. Drain lower layer and reserve for further treatment. Wash amyl alcohol ext. in rotation with 25 ml portions 5% NaCl soln until washings are colorless or nearly so. Add washings to original aq. soln. Dil. amyl alcohol ext. with equal vol. petr. ether and wash with 25 ml portions H_2O until all color is extd. Coloring matters obtained are orange I and guinea green B. For their sepn see (1) below.

Treat amyl alcohol-petr. ether soln with 10 ml portions 0.1*N* NaOH or with 10 ml portions NH_4OH (1+9), to remove erythrosine. Acidify original soln and washings (from which 3 named dyes were removed) with HCl (1 vol. acid to 40 vols soln) and ext. in 50 ml vols with three 50 ml portions amyl alcohol. Reserve lower aq. layer for further treatment. Wash amyl alcohol ext. with 25 ml portions 0.25*N* HCl until washings are colorless or nearly so. Combine washings with aq. soln above. Ext. amyl alcohol with several 25 ml portions H_2O until all color is extd. Coloring matters obtained are ponceau 3R, ponceau SX, and naphthol yellow S. For their sepn see (2).

Treat original soln and washings (from which 6 named dyes were removed) in 50 ml vol. with three 50 ml portions *glycerol dichlorohydrin*. Reserve upper aq. layer for further treatment. Wash dichlorohydrin ext. in rotation with several 20 ml portions 25% NaCl soln. Combine washings with aq. soln above. Dil. dichlorohydrin ext. with 2 vols CCl_4 and ext. with several 25 ml portions H_2O until all color is extd. Coloring matters obtained are light green SF yellowish, fast green FCF, and brilliant blue FCF. For their sepn see (3).

Further acidify original soln and washings (from which the 9 named dyes were removed) with HCl (1 vol. acid to 40 vols soln) and ext. in 50 ml vols with three 50 ml portions amyl alcohol. (If color intensity of soln was not too strong, all coloring matter should have been extd by solvent.) Discard lower colorless or nearly colorless layer and wash out dyes from amyl alcohol ext. in rotation with several 25 ml portions H_2O, until all color is extd. Coloring matters obtained are indigotine, amaranth, tartrazine, and sunset yellow FCF. For their sepn see (4).

(1) *Orange I and guinea green B.*—Ext. combined colors with two 20 ml portions glycerol dichlorohydrin. Discard colorless upper aq. layer, dil. solvent with 2 vols CCl_4, and ext. orange I in rotation with several 10 ml portions H_2O, and guinea green B with several 10 ml portions 25% alcohol.

(2) *Ponceau 3R, ponceau SX, and naphthol yellow S.*—Acidify combined colors with HCl (1 part acid to 10 parts soln) and ext. naphthol yellow S with two 20 ml portions washed EtOAc or amyl acetate. (Ponceau 3R and ponceau SX are not extd appreciably and remain in aq. layer.) Wash solvent with 5 ml portions 1*N* HCl to remove traces of the ponceaus. Remove naphthol yellow S from combined EtOAc or amyl acetate exts with 5 ml portions NH_4OH (1+9). Ext. remaining ponceau soln with 20 ml portions amyl alcohol and wash out excess of acid twice with few ml portions H_2O. Dil. amyl alcohol with equal vol. petr. ether and remove color with small vols H_2O.

Treat 10 ml of this soln with 1 ml HCl, 2 ml satd Br-H_2O, and lastly 3 ml *satd hydrazine sulfate soln;* immediately pour into test tube contg 10 ml 2*N* Na_2CO_3 and 2 drops *1% alc. α-naphthol.* (Light orange soln indicates ponceau 3R; deep brownish-red soln indicates ponceau SX.) Add 5 ml ether to soln, mix well, and drain lower aq. layer which, if colored, contains ponceau SX. To ether ext. add equal vol. HCl; formation of purplish soln confirms presence of ponceau 3R.

(3) *Light green SF yellowish, fast green FCF, and brilliant blue FCF.*—Treat combined colors with equal vol. 2*N* Na_2CO_3 and ext. in 25 ml vols with two 50 ml portions *n-butyl alcohol.* Drain lower aq. layer contg the fast green FCF and wash out last traces from solvent with 25 ml portions 2*N* Na_2CO_3. Reserve washings and add to aq. soln for confirmatory tests. Light green SF yellowish is colorless in the solvent while brilliant blue FCF imparts bluish-green color.

To prove presence of light green SF yellowish in presence of brilliant blue FCF proceed as follows: Dil. solvent with equal vol. petr. ether and remove color with small portions of H_2O. Treat

20 ml soln with 4 ml 10% NaOH soln and boil 5 min. Brilliant blue FCF is changed to red; light green SF yellowish is changed to yellow. Acidify with 10 ml HOAc, which changes brilliant blue FCF to violet and light green SF yellowish to green. Treat with ca 3 g Zn dust and heat until soln is decolorized. Filter, make slightly alk. with NH_4OH and then acid with HOAc, and bring to boil. In presence of light green SF yellowish, deep green soln is formed while brilliant blue FCF remains colorless.

(4) *Indigotine, amaranth, tartrazine, and sunset yellow FCF.*—To sep. indigotine heat to boiling small portion of soln, which should be neutral or faintly acid, and add few crystals of $Na_2S_2O_4$ until all dyes are reduced. On adding few drops HOAc and shaking with air, indigotine is quickly restored, while amaranth, tartrazine, and sunset yellow FCF are destroyed.

If positive test for indigotine is obtained, add several decigrams *urea* to remainder of mixed dye soln, heat, and while mixt. is boiling add 1 or 2 drops *10% $NaNO_2$ soln.* Indigotine is converted to pale yellow isatine sulfonate, while amaranth, tartrazine, and sunset yellow FCF are but little affected. Acidify resultant mixt. with H_2SO_4 (1+4), using 1 part dil. acid to 10 parts soln. Ext. in 25 ml portions with three 50 ml portions *n*-butyl alcohol. Drain lower layer and pass successively thru all separators. Reserve aq. layer if colored; if not colored, discard.

Prep. following soln: 13.5 ml H_2SO_4, 100 g anhyd. Na_2SO_4, and enough H_2O to make 1 L. Ext. the butyl alcohol successively with 25 ml portions of this soln until washings are colorless. Reserve them for amaranth and tartrazine. Dil. the butyl alcohol with equal vol. petr. ether and remove sunset yellow FCF with H_2O. Confirm spectrophotometrically.

Acidify reserved soln with HCl (1 vol. acid to 20 of soln) and ext. with two 30 ml portions amyl alcohol. (This exts both amaranth and tartrazine while isatine compound, being less readily extd, remains in lower layer and is discarded.) Remove coloring matter with several 10 ml portions H_2O. To portion of soln add 5 drops NH_4OH and few $Na_2S_2O_4$ crystals. (This treatment destroys amaranth completely, leaving tartrazine practically unaltered.)

Add excess of HCl and speedily ext. dye with small vol. amyl alcohol, from which soln tartrazine can be removed with 0.25*N* HCl. Treat another 10 ml portion of the neutral dye soln in test tube with 2 ml 20% NH_4Cl soln and 1 ml 25% KCN soln, and heat in boiling H_2O bath 5 min. Cool rapidly, acidify with 2 ml HCl, and ext. with 10 ml amyl alcohol (caution). Drain lower layer and discard. Remove tartrazine with 5 ml portions 0.25*N* HCl; amaranth is converted to lower sulfonated dye, and is not removed at this acid concn. Dil. solvent with equal vol. petr. ether and ext. dye with small vols H_2O (amaranth is modified to brownish-red dye).

35.009 Identification—Procedure

(a) *Oil-soluble dyes.*—Prep. soln of the isolated dye of suitable concn in $CHCl_3$. Det. spectrophotometric curve of this soln and compare this curve with those of known dyes in $CHCl_3$ solns detd on same instrument under same conditions.

(b) *Water-soluble dyes.*—Prep. ca neutral soln of the dye in concn suitable for spectrophotometric analysis with cells and instruments available. Divide soln into 3 portions and to 1 portion add few crystals of NH_4OAc. To second portion add HCl to make ca 0.1*N*. To third portion add NaOH soln to make ca 0.1*N*. Det. spectrophotometric curves of the 3 solns and compare these curves with corresponding curves of known dyes detd under same conditions on same instrument.

If spectrophotometric data of unknown color cannot be correlated with that of a known color, unknown color may be mixt. In such cases subject unknown color soln to chromatography. For oil-sol. colors, procedure of Weiss (*7*) may be used. For water-sol. colors either Tilden's paper chromatographic procedure (*8*), or following column chromatographic method may be used.

35.010 PREPARATION OF COLUMN

Tamp lightly glass wool plug into constricted end of chromatographic tube ca 40″ long×1″ diam. Prep. thin aq. slurry of ca 40 g *powd. cellulose,* such as Solka-Floc BW 40, and pour into column. Let liquid drain as cellulose settles and add more slurry as needed until all is added. When liquid level drops almost to top of adsorbent bed, add wash of 20% NaCl soln. Just before last of this soln enters adsorbent, close constricted end of column. Column may be used immediately or may be stored for several weeks before use. (Column described is adequate for 0.5–2.0 mg total dye. Column size may be varied if more or less dye is present.)

35.011 CHROMATOGRAPHIC SEPARATION

To neutral aq. soln of the color add enough NaCl to make 20% soln. Pour soln into column so that adsorbent bed is not disturbed; then open constricted end of tube. When last of soln is ready to pass into adsorbent bed, add few ml 20% NaCl soln. If any color moves down column at moderate rate, continue washing with 20% NaCl soln. If all color remains at or near top of column, change to 10% NaCl soln. If this soln fails to move any

color down column, change to 5% NaCl soln. Continue lowering NaCl concn by half until concn is found which moves color down column at moderate rate.

Continue adding appropriate concn of NaCl soln until color is eluted and collected. If color seps into 2 or more bands as it progresses down column, collect each band separately. In some cases it may be necessary to change to still more dil. NaCl soln to elute upper bands of color. If 2 or more bands of color are found, examine each spectrophotometrically as in **35.009**(**b**). If this procedure gives no indication that more than 1 color is present, it may be assumed that color is not mixt.

Natural Coloring Matters—First Action

35.012 Properties

As a class, natural coloring matters show much less tendency to dye animal fiber than do common synthetic colors. Many crude products used contain number of colored substances, and complete sepn is not practicable. As dil. solns of most of natural coloring matters are sensitive to alkalies and some are sensitive to acids, such reagents must be used with care. Relatively few good tests are known for common natural colors. Some of their most useful analytical properties (*9*) are given in Table 1, page 580.

Properties of pure prepns of various natural coloring matters are described, for most part, by Rupe (*10*), and by Perkin and Everest (*11*), reference being made in these works to original literature. Properties of chlorophylls and carotenoids are given by Willstätter and Stoll (*12*); those of coloring matters of cornflower, rose, pelargona flower, larkspur, cranberry, whortleberry, purple grape, sloe, cherry, plum, radish, and red beet by Willstätter and coworkers (*13*).

35.013 Separation

(**a**) *By extraction with ether from neutral solns.*—From neutral solns ether exts carotene, xanthophyll (pigments found in leaves, fats and oils, egg yolk, carrots, etc.), coloring matter of tomatoes and paprika, and green chlorophyll. Coloring matter remains in the ether soln on shaking with $1N$ NaOH or $1N$ HCl, no apparent change taking place, altho chemically the substances may be altered more or less by this treatment.

(**b**) *By extraction with ether from acid solns.*—From slightly acid solns ether very readily and completely exts coloring matter of alkanet, annatto, turmeric, and red dyewoods, sandalwood, camwood, and barwood. It exts, in large proportions, flavone coloring matters of fustic, Persian berries, and quercitron (after hydrolysis), as well as coloring matter of brazilwood and green derivatives formed from chlorophyll by alk. treatment. It exts, in relatively small quantity, coloring matters of logwood, orchil, saffron, and cochineal. Coloring matters of this group are readily removed from ether by shaking with alk. solns, but in most cases they rapidly undergo chemical change.

(**c**) *By extraction with amyl alcohol from acid solns.*—From slightly acid solns amyl alcohol exts major part of coloring matters of logwood, orchil, saffron, and cochineal. Amyl alcohol exts relatively small proportions of caramel and anthocyans constituting red coloring matter of most common fruits.

Identification

35.014 *By Color Changes Produced with Various Reagents*

Evap. to dryness ether solns obtained in **35.013**(**a**) and (**b**), warm residue with little alcohol, and dil. with H_2O. Dil. amyl alcohol soln obtained in **35.013**(**c**) with petr. ether and ext. with H_2O. To portions of these somewhat purified solns of coloring matter apply reagents as follows:

Hydrochloric acid.—Add to soln, first, 1 or 2 drops HCl; then excess equal to 3–4 times vol. soln.

Sodium or potassium hydroxide.—Make soln slightly alk. by adding 1 drop 10% NaOH or KOH soln. (Red color changing to yellow, especially on warming, may be due to presence of gallate antioxidants.)

Sodium hyposulfite.—Add small $Na_2S_2O_4$ crystal.

Ferric chloride.—Add small quantity of freshly prepd 0.5% $FeCl_3$ soln very carefully, small drop at time, as colors are not always obtained when excess is used.

Alum.—Add to test soln $\frac{1}{5}$ its vol. of 10% K- or NH_4-alum soln.

Uranium acetate.—Add 5% $UO_2(OAc)_2.2H_2O$ soln dropwise.

Sulfuric acid on dry color.—Evap. small quantity of soln or of coloring matter in porcelain dish. Cool thoroly and treat dry residue with 1 or 2 drops cold H_2SO_4. Colors are sometimes extremely transitory, and may be observed only instant acid wets residue.

Table 1 shows behavior of certain natural coloring matters when treated in manner described above.

35.015 *By Special Tests*

(a) *Chlorophyll.*—"Brown phase reaction" (*14*) may be useful to characterize chlorophyll, when

TABLE 1.—*Reaction of certain natural coloring matters to common reagents*

COLORING MATTER	HYDROCHLORIC ACID	10% SODIUM HYDROXIDE SOLUTION	SODIUM HYPOSULFITE	0.5% FERRIC CHLORIDE SOLUTION	10% ALUM SOLUTION	5% URANIUM ACETATE SOLUTION	SULFURIC ACID ON DRY COLOR
Logwood	Deep red with excess of acid	Violet to violet-blue	Almost decolorized, color returning imperfectly by reoxidation	Dark shades of violet, brown, or black (the first hue often evanescent)	Rose-red (change rather slow)	Violet, quickly fading	Red, changing to yellow
Red woods (brazilwood, sandalwood, camwood and barwood)	Deep red with excess of acid	Violet-red	. . .	Dark shades of violet, brown, or black (the first hue often evanescent	Rose-red (change rather slow)		
Anthocyans of red fruit juices		Change to green, dull blue, or slate color, usually very quickly becoming browner by oxidation	Anthocyanidins derived by hydrolysis, almost completely decolorized	.			
Alkanet		Deep blue	. . . . .	.		Yellowish green	Violet-blue
Orseil	Little or no change	Blue	Decolorized, color returning when shaken with air. Reaction more easily seen in alk. soln			. .	Violet-blue
Cochineal	Little or no change	Violet	No marked change	Slightly darker		Green	
Annatto	Remains orange. Little change	.	Little affected	No marked change. Perhaps somewhat browner	. . .	.	Blue
Turmeric (soln in ether or alcohol characterized by pure yellow color and light green fluorescence)	Orange-red or carmine-red on addn of several vols of concd acid	Orange-brown	Little affected	No marked change. Perhaps somewhat browner	Little change	Somewhat browner	Red
Flavone colors of fustic, Persian berries, quercitron, etc.	Becomes intensely yellow with 2–4 vols concd acid	Bright yellow	Little affected	Olive-green or black colorations	More strongly yellow; fustic, developing green fluorescence	Orange colorations	Yellow to orange
Saffron	Little or no change	Remains yellow	Little affected	No marked change. Perhaps somewhat browner	Little change	Not affected	Blue
Carotene and xanthophyll	Little change. Perhaps slightly paler	Little or no change	Little affected	. . .	.	. . .	Blue, reaction obtained with difficulty
Chlorophyll	More brownish	"Brown phase reaction" 35.015(a)	.			. .	. . . .
Caramel	Little or no change	Little change or slightly deeper brown	Slightly paler	No change	. . .		

it has not been previously treated with alkalies. Treat green ether or petr. ether soln of coloring matter with small quantity of 10% soln KOH in MeOH. Color becomes brown, quickly returning to green.

(b) *Annatto (15).*—Pour on moistened filter alk. soln of color obtained by shaking out oil or melted and filtered fat with warm 2% NaOH soln. If annatto is present, paper absorbs color, so that when washed with gentle stream of H_2O it remains dyed straw color. Dry filter, add drop of 40% $SnCl_2$ soln, and again dry carefully. If color turns purple, presence of annatto is confirmed.

(c) *Turmeric.*—Treat aq. or dil. alc. soln of color with HCl until shade just begins to appear slightly orange. Divide mixt. into 2 parts and add some H_3BO_3 powder or crystals to one portion. Marked reddening is quickly apparent, best seen by comparison with portion to which the H_3BO_3 has not been added. Test may also be made by dipping piece of filter paper in alc. soln of coloring matter, drying at 100°, and then moistening with weak soln of H_3BO_3 to which few drops of HCl have been added. On drying again, cherry-red color is developed.

(d) *Cochineal.*—When presence of cochineal is suspected, acidify mixt. with $\frac{1}{3}$ its vol. HCl and shake with amyl alcohol. Wash amyl alcohol soln of coloring matter 2–4 times with equal vols H_2O to remove HCl, etc. Dil. amyl alcohol with 1–2 vols petr. ether and shake with few small portions of H_2O to remove color. Divide combined aq. exts into 2 portions.

To first add, dropwise, 5% $UO_2(OAc)_2.2H_2O$ soln, shaking thoroly after each addn. In presence of cochineal characteristic emerald-green color is produced *(16)*. Green color with U salts is not developed in presence of much free acid. Therefore, add little NaOAc before making this test, or correspondingly larger quantity of the $UO_2(OAc)_2$ soln must be added.

To second portion add 1 or 2 drops NH_4OH; in presence of cochineal, violet color results. This, however, is not so characteristic as first test, as many fruit colors give almost identical reactions. Cochineal is not decolorized by $Na_2S_2O_4$ in either acid, neutral, or alk. soln (differs from orchil).

As cochineal lakes often contain Sn, always make further examination for this metal when H_2O-insol. cochineal compounds seem to be present.

(e) *Orchil.*—This coloring matter is either sulfonated or unsulfonated. Unsulfonated orchil is readily extd by amyl alcohol from weak acid soln, while extn of sulfonated color is incomplete even from strongly acidified soln. Behavior of color towards acids and alkalies is similar to cochineal, *e.g.,* HCl produces yellow shade and alkalies produce bluish shade. $Na_2S_2O_4$ reduces orchil, but color is restored by air oxidation (differing from cochineal). Characteristic property of orchil is to dye, strip, and redye wool readily.

(f) *Caramel.*—Number of tests have been developed for this coloring matter, most of them based upon insolubility in ether, $CHCl_3$, or amyl alcohol. Probably most sensitive test is Woodman-Newhall *(17)* modification of Amthor test with slight deviation.

To 10–20 ml neutral soln of the color in small centrifuge tube add 2 ml 5% $ZnCl_2$ soln and 2 ml 2% KOH soln, stir well, and centrifuge. Pour off liquid, and add 25 ml boiling H_2O to magma. Mix, centrifuge, and pour off liquid. Repeat this operation until aq. wash liquid is colorless. Dissolve ppt with 15 ml 10% HOAc, conc., neutralize carefully, and filter. Divide into 2 portions. To one add 3–5 vols *paraldehyde* in 50 ml g-s. cylinder, and just enough absolute alcohol to form homogeneous soln (avoid excess). Caramel is indicated by formation of brownish ppt on standing. To other portion of caramel soln add equal vol. *freshly prepd reagent* consisting of phenylhydrazine.HCl, 2 parts; $NaOAc.3H_2O$, 3 parts; H_2O, 20 parts. Dark brown ppt is formed in presence of caramel.

ANALYSIS OF COMMERCIAL COAL-TAR COLORS

35.016 Specifications for Certifiable Coal-Tar Colors

Federal Food, Drug, and Cosmetic Act provides for listing of coal-tar colors that are harmless and suitable for use in foods, drugs, or cosmetics, and for certification of batches of those colors. "Compilation of Regulations for Color Certification," May 1959, lists these certifiable colors, together with specifications for identity and purity to which they must conform in order to be certified. Certifiable coal-tar colors, and detns that must be made on each to establish compliance with specifications, are listed below. Methods to be used for these detns are noted by number:

(a) *Determinations to be made on all straight colors.*—Det. Pb as in **35.096**, **35.099**, or **35.100**.

(b) *Straight Colors—Foods, Drugs, and Cosmetics*

FD&C Blue No. 1 (Brilliant Blue FCF)

	Method
Volatile matter (135°)	35.035
H_2O-insol. matter	35.037
Ether exts	35.054
$NaCl+Na_2SO_4$	35.115 & 35.119
NaOAc	35.123
Mixed oxides	35.102
Pure dye	35.020(c)

FD&C Blue No. 2 (Indigotine)

	Method
Volatile matter (135°)	35.035
H_2O-insol. matter	35.037
Ether exts	35.055
$NaCl+Na_2SO_4$	35.115 & 35.119
Mixed oxides	35.102
Pure dye	35.020(c)

FD&C Green No. 1 (Guinea Green B)

	Method
Volatile matter (135°)	35.035
H_2O-insol. matter	35.037
Ether exts	35.055
$NaCl+Na_2SO_4$	35.115 & 35.119
Mixed oxides	35.102
Pure dye	35.020(c)

FD&C Green No. 2 (Light Green SF Yellowish)

	Method
Volatile matter (135°)	35.035
H_2O-insol. matter	35.037
Ether exts	35.054
$NaCl+Na_2SO_4$	35.115 & 35.119
Mixed oxides	35.102
Pure dye	35.020(c)

FD&C Green No. 3 (Fast Green FCF)

	Method
Volatile matter (135°)	35.035
H_2O-insol. matter	35.037
Ether exts	35.054
$NaCl+Na_2SO_4$	35.115 & 35.119
Mixed oxides	35.102
Pure dye	35.020(c)

Citrus Red No. 2 (For coloring oranges only)

	Method
Volatile matter (100°)	35.035
Sulfated ash	35.101
H_2O-sol. matter	35.124
Matter sol. in CCl_4	35.039
Pure dye	35.028 & 35.031

FD&C Red No. 1 (Ponceau 3R)

	Method
Volatile matter (135°)	35.035
H_2O-insol. matter	35.037
Ether exts	35.054
$NaCl+Na_2SO_4$	35.115 & 35.119
Mixed oxides	35.102
Pure dye	35.020(a)

FD&C Red No. 2 (Amaranth)

	Method
Volatile matter (135°)	35.035
H_2O-insol. matter	35.037
Ether exts	35.054
$NaCl+Na_2SO_4$	35.115 & 35.119
Mixed oxides	35.102
Pure dye	35.020(a)

FD&C Red No. 3 (Erythrosine)

	Method
Volatile matter (135°)	35.035
H_2O-insol. matter	35.037
Ether exts	35.054(a) & (b)
$NaCl+Na_2SO_4$	35.120 & 35.119
NaI	35.120
Mixed oxides	35.102
Organically combd I in pure dye, free from H_2O of crystn	35.104
Pure dye	35.021(a)

FD&C Red No. 4 (Ponceau SX)

	Method
Volatile matter (135°)	35.035
H_2O-insol. matter	35.037
Ether exts	35.054
$NaCl+Na_2SO_4$	35.115 & 35.119
Mixed oxides	35.102
Pure dye	35.020(c)

FD&C Violet No. 1 (Wool Violet 5BN, Acid Violet 6B)

	Method
Volatile matter (135°)	35.035
H_2O-insol. matter	35.037
Ether exts	35.055
$NaCl+Na_2SO_4$	35.115 & 35.119
Mixed oxides	35.102
Pure dye	35.020(c) *or* (e)

FD&C Yellow No. 5 (Tartrazine)

	Method
Volatile matter (135°)	35.035
H_2O-insol. matter	35.037
Ether exts	35.054
$NaCl+Na_2SO_4$	35.115 & 35.119
Mixed oxides	35.102
Subsidiary dye	35.081
Pure dye	35.020(c)

FD&C Yellow No. 6 (Sunset Yellow FCF)

	Method
Volatile matter (135°)	35.035
H_2O-insol. matter	35.037
Ether exts	35.054
$NaCl+Na_2SO_4$	35.115 & 35.119
Mixed oxides	35.102
Subsidiary dye	35.084
Pure dye	35.020(a)

Lakes

	Method
Ether exts	35.051
Sol. chlorides+sulfates (as Na salts)	*See individual colors*
Intermediates	*See individual colors*

(c) *Straight Colors—Drugs and Cosmetics*

D&C Black No. 1 (Naphthol Blue Black)

	Method
Volatile matter (135°)	35.035
H_2O-insol. matter	35.037
Ether exts	35.055
Aniline	35.059
p-Nitroaniline	35.060
$NaCl+Na_2SO_4$	35.115 & 35.119
Mixed oxides	35.102
Pure dye	35.020(c)

D&C Blue No. 4 (Alphazurine FG)

	Method
Volatile matter (135°)	35.035
H_2O-insol. matter	35.037
Ether exts	35.054
$NH_4Cl+(NH_4)_2SO_4$	35.115 & 35.119
Mixed oxides	35.102
Pure dye	35.020(c)

D&C Blue No. 5 (Alizarin Astrol B)

	Method
Volatile matter (135°)	35.035
H_2O-insol. matter	35.037
Ether exts	35.054
$NaCl+Na_2SO_4$	35.115 & 35.119
Mixed oxides	35.102
Pure dye	35.024 & 35.027

D&C Blue No. 6 (Indigo)

	Method
Volatile matter (135°)	35.035
Sulfated ash	35.101
Ether exts	35.051
Pure dye	35.020(g)

D&C Blue No. 7 (Patent Blue NA)

	Method
Volatile matter (135°)	35.035
H_2O-insol. matter	35.037
Ether exts	35.054
$NaCl+Na_2SO_4$	35.115 & 35.119
Mixed oxides	35.102
Pure dye	35.020(c)

D&C Blue No. 8 (Patent Blue CA)

	Method
Volatile matter (135°)	35.035
H_2O-insol. matter	35.037
Ether exts	35.054
Chlorides and sulfates (Ca salts)	35.115 & 35.119
$Fe_2O_3+Al_2O_3$	35.102
Pure dye	35.020(c)

D&C Blue No. 9 (Carbanthrene Blue)

	Method
Volatile matter (135°)	35.035
Sulfated ash	35.101
Matter extractable by alc. HCl	35.052
Organically combd Cl in pure dye	35.107
Pure dye	35.024

D&C Brown No. 1 (Resorcin Brown)

	Method
Volatile matter (135°)	35.035
H_2O-insol. matter	35.037
Ether exts	35.055
m-Xylidine	35.059
$NaCl+Na_2SO_4$	35.115 & 35.119
Mixed oxides	35.102
Pure dye	35.020(c)

D&C Green No. 4 (Light Green CF Yellowish)

	Method
Volatile matter (135°)	35.035
H_2O-insol. matter	35.037
Ether exts	35.054
Chlorides and sulfates (as Ca salts)	35.115 & 35.119
$Fe_2O_3+Al_2O_3$	35.102
Pure dye	35.020(c)

D&C Green No. 5 (Alizarin Cyanine Green F)

	Method
Volatile matter (135°)	35.035
H_2O-insol. matter	35.037
Ether exts	35.055
$NaCl+Na_2SO_4$	35.115 & 35.119
Mixed oxides	35.102
Pure dye	35.020(b)

D&C Green No. 6 (Quinizarin Green SS)

	Method
Volatile matter (135°)	35.035
Sulfated ash	35.101
H_2O-sol. matter	35.124
Matter insol. in CCl_4	35.048
Pure dye	35.031
M.p.	35.128

D&C Green No. 7 (Acid Fast Green)

	Method
Volatile matter (135°)	35.035
H_2O-insol. matter	35.037
Ether exts	35.055
$NaCl+Na_2SO_4$	35.115 & 35.119
Mixed oxides	35.102
Pure dye	35.020(c)

D&C Green No. 8
(Pyranine Concentrated)

	Method
Volatile matter (135°)	35.035
H_2O-insol. matter	35.037
$CHCl_3$ ext.	35.125
Pyrene	35.071
$NaCl+Na_2SO_4$	35.115 & 35.119
Mixed oxides	35.102
Pure dye	35.025 *or* 35.027

D&C Orange No. 3
(Orange G)

	Method
Volatile matter (135°)	35.035
H_2O-insol. matter	35.037
Ether exts	35.054
Aniline	35.059
$NaCl+Na_2SO_4$	35.115 & 35.119
Mixed oxides	35.102
Pure dye	35.020(c)

D&C Orange No. 4
(Orange II)

	Method
Volatile matter (135°)	35.035
H_2O-insol. matter	35.037
Ether exts	35.054
β-Naphthol	35.067(a)
$NaCl+Na_2SO_4$	35.115 & 35.119
Mixed oxides	35.102
Pure dye	35.020(c)

D&C Orange No. 5
(Dibromofluorescein)

	Method
Volatile matter (135°)	35.035
Insol. matter (alk. soln)	35.050
Ether exts (alk. soln)	35.056
NaCl	35.120
Mixed oxides	35.102
Free Br	35.112
Organically combd Br in pure dye	35.106
Pure dye	35.021(b)

D&C Orange No. 6
(Dibromofluorescein NA)

	Method
Volatile matter (135°)	35.035
H_2O-insol. matter	35.037
Ether exts	35.055(a) & (b)
$NaCl+Na_2SO_4$	35.120 & 35.119
Mixed oxides	35.102
Free Br	35.112
Organically combd Br in pure dye	35.106
Pure dye	35.021(a)

D&C Orange No. 7
(Dibromofluorescein K)

	Method
Volatile matter (135°)	35.035
H_2O-insol. matter	35.037
Ether exts	35.055(a) & (b)
$KCl+K_2SO_4$	35.120 & 35.119
Mixed oxides	35.102
Free Br	35.112
Organically combd Br in pure dye	35.106
Pure dye	35.021(a)

D&C Orange No. 8
(Dichlorofluorescein)

	Method
Volatile matter (135°)	35.035
Insol. matter (alk. soln)	35.050
Ether exts (alk. soln)	35.056
$NaCl+Na_2SO_4$	35.120 & 35.119
Mixed oxides	35.102
Free Cl	35.112
Organically combd Cl in pure dye	35.107
Pure dye	35.020(e)

D&C Orange No. 9
(Dichlorofluo escein NA)

	Method
Volatile matter (135°)	35.035
H_2O-insol. matter	35.037
Ether exts	35.055(a) & (b)
$NaCl+Na_2SO_4$	35.120 & 35.119
Mixed oxides	35.102
Free Cl	35.112
Organically combd Cl in pure dye	35.107
Pure dye	35.020(e)

D&C Orange No. 10
(Diiodofluorescein)

	Method
Volatile matter (135°)	35.035
Insol. matter (alk. soln)	35.050
Ether exts (alk. soln)	35.056
NaCl	35.120
Mixed oxides	35.102
Organically combd I in pure dye	35.104
Pure dye	35.021(b)

D&C Orange No. 11
(Erythrosine Yellowish NA)

	Method
Volatile matter (135°)	35.035
H_2O-insol. matter	35.037
Ether exts	35.055(a) & (b)
$NaCl+Na_2SO_4$	35.120 & 35.119
Mixed oxides	35.102
Organically combd I in pure dye	35.104
Pure dye	35.021(a)

D&C Orange No. 12
(Erythrosine Yellowish K)

	Method
Volatile matter (135°)	35.035
H_2O-insol. matter	35.037
Ether exts	35.055(a) & (b)
$KCl+K_2SO_4$	35.120 & 35.119
Mixed oxides	35.102
Organically combd I in pure dye	35.104
Pure dye	35.021(a)

D&C Orange No. 13
(Erythrosine Yellowish NH)

	Method
Volatile matter (135°)	35.035
H_2O-insol. matter	35.037
Ether exts	35.055(a) & (b)
$NH_4Cl+(NH_4)_2SO_4$	35.120 & 35.119
Mixed oxides	35.102
Organically combd I in pure dye	35.104
Pure dye	35.021(a)

D&C Orange No. 14
(Orange TR)

	Method
Volatile matter (135°)	35.035
Sulfated ash	35.101
Insol. matter (alk. soln)	35.050
Sol. matter (in 1% aq. HCl)	35.126
Free Br	35.112
Organically combd Br in pure dye	35.106
Pure dye	35.021(b)

D&C Orange No. 15
(Alizarin)

	Method
Volatile matter (135°)	35.035
H_2O-sol. matter	35.124
Ether-insol. matter	35.047
NaCl	35.115
Mixed oxides	35.102
Pure dye	35.032
M.p.	35.128

D&C Orange No. 16
(Dibromodiiodofluorescein)

	Method
Volatile matter (135°)	35.035
Insol. matter (alk. soln)	35.050
Ether exts (alk. soln)	35.056
NaCl	35.120
Mixed oxides	35.102
Free halogens	35.112
Organically combd Br in pure dye	35.106
Organically combd I in pure dye	35.104
Pure dye	35.021(b)

D&C Orange No. 17
(Permatone Orange, Permanent Orange)

	Method
Volatile matter (135°)	35.035
Sulfated ash	35.101
Matter insol. in toluene	35.044
β-Naphthol	35.067(c) or 35.064
Pure dye	35.031

D&C Red No. 5
(Ponceau 2R)

	Method
Volatile matter (135°)	35.035
H_2O-insol. matter	35.037
Ether exts	35.054
Xylidine	35.059
$NaCl+Na_2SO_4$	35.115 & 35.119
Mixed oxides	35.102
Pure dye	35.020(a)

D&C Red No. 6
(Lithol Rubin B)

	Method
Volatile matter (135°)	35.035
H_2O-insol. matter	35.037
Ether exts (isopropyl ether)	35.051
$NaCl+Na_2SO_4$	35.115 & 35.119
Mixed oxides	35.102
Pure dye	35.020(c)

D&C Red No. 7
(Lithol Rubin BCA)

	Method
Volatile matter (135°)	35.035
Ether exts (isopropyl ether)	35.051
Chlorides and sulfates (as Ca salts)	35.115 & 35.119
$Fe_2O_3+Al_2O_3$	35.102
Pure dye	35.020(f)

D&C Red No. 8
(Lake Red C)

	Method
Volatile matter (135°)	35.035
Ether exts (isopropyl ether)	35.051
β-Naphthol	35.067(c)
$NaCl+Na_2SO_4$	35.115 & 35.119
Mixed oxides	35.102
Lake Red C Amine	35.062
Pure dye	35.020(f)

D&C Red No. 9
(Lake Red CBA)

	Method
Volatile matter (135°)	35.035
Ether exts (isopropyl ether)	35.051
β-Naphthol	35.067(c)
$NaCl+Na_2SO_4$	35.115 & 35.119
$Fe_2O_3+Al_2O_3$	35.102
Lake Red C Amine	35.062
Pure dye	35.020(f)

D&C Red No. 10)
(Lithol Red)

	Method
Volatile matter (135°)	35.035
Ether exts (isopropyl ether)	35.051
β-Naphthol	35.067(c)
$NaCl+Na_2SO_4$	35.115 & 35.119
Mixed oxides	35.102
Pure dye	35.020(f)

D&C Red No. 11
(Lithol Red CA)

	Method
Volatile matter (135°)	35.035
Ether exts (isopropyl ether)	35.051
β-Naphthol	35.067(c)
Chlorides & sulfates (as Ca salts)	35.115 & 35.119
$Fe_2O_3+Al_2O_3$	35.102
Pure dye	35.020(f)

D&C Red No. 12
(Lithol Red BA)

	Method
Volatile matter (135°)	35.035
Ether exts (isopropyl ether)	35.051
β-Naphthol	35.067(c)
$NaCl+Na_2SO_4$	35.115 & 35.119
$Fe_2O_3+Al_2O_3$	35.102
Pure dye	35.020(f)

D&C Red No. 13
(Lithol Red SR)

	Method
Volatile matter (135°)	35.035
Ether exts (isopropyl ether)	35.051
β-Naphthol	35.067(c)
$NaCl+Na_2SO_4$	35.115 & 35.119
$Fe_2O_3+Al_2O_3$	35.102
Pure dye	35.020(f)

D&C Red No. 14
(Lake Red D)

	Method
Volatile matter (135°)	35.035
Ether exts (isopropyl ether)	35.051
β-Naphthol	35.067(c)
$NaCl+Na_2SO_4$	35.115 & 35.119
Mixed oxides	35.102
Pure dye	35.020(f)

D&C Red No. 15
(Lake Red DBA)

	Method
Volatile matter (135°)	35.035
Ether exts (isopropyl ether)	35.051
β-Naphthol	35.067(c)
NaCl+Na_2SO_4	35.115 & 35.119
Fe_2O_3+Al_2O_3	35.102
Pure dye	35.020(f)

D&C Red No. 16
(Lake Red DCA)

Volatile matter (135°)	35.035
Ether exts (isopropyl ether)	35.051
β-Naphthol	35.067(c)
Chlorides & Sulfates (as Ca salts)	35.115 & 35.119
Fe_2O_3+Al_2O_3	35.102
Pure dye	35.020(f)

D&C Red No. 17
(Toney Red)

Volatile matter (135°)	35.035
Insol. matter (in toluene)	35.010
Aniline	35.059
β-Naphthol	35.067(b)
NaCl	35.115
Mixed oxides	35.102
Pure dye	35.020(e) or 35.031

D&C Red No. 18
(Oil Red OS)

Volatile matter (100°)	35.035
Sulfated ash	35.101
H_2O-sol. matter	35.124
Matter insol. in CCl_4	35.039
NaCl+Na_2SO_4	35.115 & 35.119
Xylidine	35.059
β-Naphthol	35.067(b)
Pure dye	35.020(e) *or* 35.031

D&C Red No. 19
(Rhodamine B)

Volatile matter (135°)	35.035
H_2O-insol. matter	35.037
Ether exts (acid soln)	35.055(c)
NaCl+Na_2SO_4	35.117 & 35.119
Mixed oxides	35.102
Pure dye	35.031

D&C Red No. 20
(Rhodamine B Acetate)

Volatile matter (135°)	35.035
H_2O-insol. matter	35.037
Ether exts from acid soln	35.054(c)
Mixed oxides	35.102
Pure dye	35.031

D&C Red No. 21
(Tetrabromofluorescein)

Volatile matter (135°)	35.035
Insol. matter (alk. soln)	35.050
Ether exts (alk. soln)	35.056
NaCl+Na_2SO_4	35.120 & 35.119
Mixed oxides	35.102
Free Br	35.112
Organically combd Br in pure dye	35.106
Pure dye	35.021(b)

D&C Red No. 22
(Eosin YS)

Volatile matter (135°)	35.035
H_2O-insol. matter	35.037
Ether exts	35.055(a) & (b)
NaCl+Na_2SO_4	35.120 & 35.119
Mixed oxides	35.102
Free Br	35.112
Organically combd Br in pure dye	35.106
Pure dye	35.021(a)

D&C Red No. 23
(Eosin YSK)

Volatile matter (135°)	35.035
H_2O-insol. matter	35.037
Ether exts	35.055(a) & (b)
KCl+K_2SO_4	35.120 & 35.119
Mixed oxides	35.102
Free Br	35.112
Organically combd Br in pure dye (free from H_2O or alcohol of crystn)	35.106
Pure dye	35.021(a)

D&C Red No. 24
(Tetrachlorofluorescein)

	Method
Volatile matter (135°)	35.035
Insol. matter (alk. soln)	35.050
Ether exts (alk. soln)	35.056
NaCl+Na_2SO_4	35.120 & 35.119
Mixed oxides	35.102
Free Cl	35.112
Organically combd Cl in pure dye	35.107
Pure dye	35.021(b)

D&C Red No. 25
(Tetrachlorofluorescein NA)

Volatile matter (135°)	35.035
H_2O-insol. matter	35.037
Ether exts	35.055(a) & (b)
NaCl+Na_2SO_4	35.120 & 35.119
Mixed oxides	35.102
Free Cl	35.112
Organically combd Cl in pure dye	35.107
Pure dye	35.021(a)

D&C Red No. 26
(Tetrachlorofluorescein K)

Volatile matter (135°)	35.035
H_2O-insol. matter	35.037
Ether exts	35.055(a) & (b)
KCl+K_2SO_4	35.120 & 35.119
Mixed oxides	35.120
Free Cl	35.112
Organically combd Cl in pure dye	35.107
Pure dye	35.021(a)

D&C Red No. 27
(Tetrachlorotetrabromofluorescein)

Volatile matter (135°)	35.035
Insol. matter (alk. soln)	35.050
Ether exts (alk. soln)	35.056(b)
NaCl	35.120
Mixed oxides	35.102
Free halogens	35.112
Organically combd Br in pure dye	35.106
Organically combd Cl in pure dye	35.108
Pure dye	35.021(b)

D&C Red No. 28
(Phloxine B)

Volatile matter (135°)	35.035
H_2O-insol. matter	35.037
Ether exts	35.055(a) & (b)
NaCl+Na_2SO_4	35.120 & 35.119
Mixed oxides	35.102
Free halogens	35.112
Organically combd Br in pure dye	35.106
Organically combd Cl in pure dye	35.108
Pure dye	35.021(a)

D&C Red No. 29
(Bluish Orange TR)

Volatile matter (135°)	35.035
Sulfated ash	35.101
Insol. matter (alk. soln)	35.050
Sol. matter (in 1% aq. HCl)	35.126
Free Br	35.112
Organically combd Br in pure dye	35.106
Pure dye	35.021(b)

D&C Red No. 30
(Helindone Pink CN)

Volatile matter (135°)	35.035
Matter insol. in xylene	35.049
NaCl	35.115
Mixed oxides	35.102
Pure dye	35.020(g)

D&C Red No. 31
(Brilliant Lake Red R)

Volatile matter (135°)	35.035
Ether ext. (isopropyl ether)	35.051
Aniline	35.059
Chlorides & sulfates (as Ca salts)	35.115 & 35.119
Fe_2O_3+Al_2O_3	35.102
Pure dye	35.020(f)

D&C Red No. 33
(Acid Fuchsin D, Naphthalene Red B)

	Method
Volatile matter (135°)	35.035
H_2O-insol. matter	35.037
Ether exts	35.054
Aniline	35.059
NaCl+Na_2SO_4	35.115 & 35.119
Mixed oxides	35.102
Pure dye	35.020(c)

D&C Red No. 34
(Deep Maroon, Fanchon Maroon)

Volatile matter (135°)	35.035
Ether exts	35.051
Cl & sulfates (as Ca salts)	35.115 & 35.119
Fe_2O_3+Al_2O_3	35.102
Pure dye	35.020(f)

D&C Red No. 35
(Toluidine Red)

Volatile matter (135°)	35.035
Sulfated ash	35.101
Matter insol. in toluene	35.044
2-Nitro-*p*-toluidine	35.060
β-Naphthol	35.067(c) *or* 35.064
Pure dye	35.031
M.p.	35.128

D&C Red No. 36
(Flaming Red)

Volatile matter (135°)	35.035
Sulfated ash	35.101
Matter insol. in toluene	35.044
o-Chloro-*p*-nitroaniline	35.060
β-Naphthol	35.067(c)
Pure dye	35.031

D&C Red No. 37
(Rhodamine B Stearate)

Volatile matter (80°)	35.035
Sulfated ash	35.101
Matter insol. in benzene	35.041
Pure dye	35.031

D&C Red No. 38
(Deep Red, Maroon)

Volatile matter (135°)	35.035
Sulfated ash	35.101
Matter insol. in alk. dioxane	35.043
2-Nitro-*p*-toluidine	35.060
Pure dye	35.024

D&C Red No. 39
(Alba Red)

Volatile matter (100°)	35.035
Matter insol. in acetone	35.045
Ether ext. (petr. ether)	35.057
Sulfated ash	35.101
Pure dye	35.020(e)

D&C Violet No. 2
(D&C Blue No. 3, Alizurol Purple SS)

Volatile matter (135°)	35.035
Sulfated ash	35.101
Matter insol. in CCl_4	35.048
p-Toluidine	35.059
Pure dye	35.031
M.p.	35.128

D&C Yellow No. 7
(Fluorescein)

Volatile matter (135°)	35.035
Insol. matter (alk. soln)	35.050
Ether ext. (alk. soln)	35.056
NaCl+Na_2SO_4	35.115 & 35.119
Mixed oxides	35.102
Pure dye	35.020(e) *or* 35.031

D&C Yellow No. 8
(Uranine)

Volatile matter (135°)	35.035
H_2O-insol. matter	35.037
Ether exts	35.055(a) & (b)
NaCl+Na_2SO_4	35.115 & 35.119
Mixed oxides	35.102
Pure dye	35.020(e) *or* 35.031

D&C Yellow No. 9
(Uranine K)

	Method
Volatile matter (135°)	35.035
H_2O-insol. matter	35.037
Ether exts	35.055(a) & (b)
$KCl+K_2SO_4$	35.115 & 35.119
Mixed oxides	35.102
Pure dye	35.020(e) *or* 35.031

D&C Yellow No. 10
(Quinoline Yellow WS)

	Method
Volatile matter (135°)	35.035
H_2O-insol. matter	35.037
Ether exts	35.054
$NaCl+Na_2SO_4$	35.115 & 35.119
Mixed oxides	35.102
Pure dye	35.024(a) & 35.027

D&C Yellow No. 11
(Quinoline Yellow SS)

	Method
Volatile matter (135°)	35.035
Sulfated ash	35.101
Matter insol. in alcohol	35.046
Pure dye	35.031
M.p.	35.128

Lakes

Ether exts	35.051
Sol. chlorides & sulfates (as Na salts)	*See individual colors*
Intermediates	*See individual colors*

(d) *Straight Colors—Externally Applied Drugs and Cosmetics*

Ext. D&C Black No. 1
(Coomassie Fast Black, Fast Black BB)

	Method
Volatile matter (135°)	35.035
H_2O-insol. matter	35.037
Ether exts	35.055
$NaCl+Na_2SO_4$	35.115 & 35.119
Mixed oxides	35.102
Pure dye	35.020(c)

Ext. D&C Blue No. 1
(Methylene Blue)

	Method
Volatile matter (110°)	35.035
H_2O-insol. matter	35.037
Ether exts	35.054
$NaCl+Na_2SO_4$	35.117 & 35.119
Mixed oxides	35.102
Pure dye	35.020(c)

Ext. D&C Blue No. 2
(Methylene Blue Zinc Double Chloride)

	Method
Volatile matter (110°)	35.035
H_2O-insol. matter	35.037
Ether exts	35.054
$NaCl+Na_2SO_4$	35.117 & 35.119
$Fe_2O_3+Al_2O_3$	35.102
Pure dye	35.020(c)

Ext. D&C Blue No. 3
(Erioglaucine X)

	Method
Volatile matter (135°)	35.035
H_2O-insol. matter	35.037
Ether exts	35.054
$NaCl+Na_2SO_4$	35.115 & 35.119
Mixed oxides	35.102
Pure dye	35.020(c)

Ext. D&C Blue No. 4
(Alizarin Saphirol)

	Method
Volatile matter (135°)	35.035
H_2O-insol. matter	35.037
Ether exts	35.054
$NaCl+Na_2SO_4$	35.115 & 35.119
Mixed oxides	35.102
Pure dye	35.024 & 34.027

Ext. D&C Blue No. 5
(Hexyl Blue)

	Method
Volatile matter (100°)	35.035
Insol. matter in CCl_4	35.039
Sulfated ash	35.101
Pure dye	35.031

Ext. D&C Green No. 1
(Naphthol Green B)

	Method
Volatile matter (135°)	35.035
Ether exts	35.054
H_2O-insol. matter	35.037
$NaCl+Na_2SO_4$	35.115 & 35.119
Pure dye	35.020(c)

Ext. D&C Orange No. 1
(Fanchon Orange, Hansa Orange)

	Method
Volatile matter (135°)	35.035
Sulfated ash	35.101
Matter insol. in toluene	35.044
2-Nitro-*p*-anisidine	35.060
Pure dye	35.031
M.p.	35.128

Ext. D&C Orange No. 2
(Indelible Orange)

	Method
Volatile matter (100°)	35.035
Ether ext. (alk. soln)	35.056
Sulfated ash	35.101
Organically combd N in pure dye	35.024
Insol. matter (alk. soln)	35.050
Pure dye	35.021(b)

Ext. D&C Orange No. 3
(Orange I)

	Method
Volatile matter (135°)	35.035
H_2O-insol. matter	35.037
Ether exts	35.054
α-Naphthol	35.070
$NaCl+Na_2SO_4$	35.115 & 35.119
Mixed oxides	35.102
Orange II	35.079
Pure dye	35.020(c)

Ext. D&C Orange No. 4
(Orange SS)

	Method
Volatile matter (100°)	35.035
Sulfated ash	35.101
H_2O-sol. matter	35.124
Matter insol. in CCl_4	35.039
o-Toluidine	35.059
β-Naphthol	35.067(b)
Pure dye	35.020(c)
M.p.	35.128

Ext. D&C Red No. 1
(Amidonaphthol Red 6B)

	Method
Volatile matter (135°)	35.035
H_2O-insol. matter	35.037
Ether exts	35.054
$NaCl+Na_2SO_4$	35.115 & 35.119
Mixed oxides	35.102
Pure dye	35.020(c)

Ext. D&C Red No. 2
(Pigment Scarlet NA)

	Method
Volatile matter (135°)	35.035
H_2O-insol matter	35.037
Ether exts	35.054
$NaCl+Na_2SO_4$	35.115 & 35.119
Mixed oxides	35.102
Pure dye	35.020(c)

Ext. D&C Red No. 3
(Violamine R)

	Method
Volatile matter (135°)	35.035
H_2O-insol. matter	35.037
Ether exts	35.055
o-Toluidine	35.059
$NaCl+Na_2SO_4$	35.115 & 35.119
Mixed oxides	35.102
Pure dye	35.020(c)

Ext. D&C Red No. 4
(Dichlorotetraiodofluorescein)

	Method
Volatile matter (135°)	35.035
Insol. matter (alk. soln)	35.050
Ether ext. (alk. soln)	35.056
NaCl	35.120
Mixed oxides	35.102
Free halogens	35.112
Organically combd I in pure dye	35.104
Organically combd Cl in pure dye	35.107
Pure dye	35.021(b)

Ext. D&C Red No. 5
(Rose Bengale TD)

	Method
Volatile matter (135°)	35.035
H_2O-insol. matter	35.037
Ether exts	35.055(a) & (b)
$NaCl+Na_2SO_4$	35.120 & 35.119
Mixed oxides	35.102
Free halogens	35.112
Organically combd I in pure dye	35.104
Organically combd Cl in pure dye	35.107
Pure dye	35.021(a)

Ext. D&C Red No. 6
(Rose Bengale TDK)

	Method
Volatile matter (135°)	35.035
H_2O-insol. matter	35.037
Ether exts	35.055(a) & (b)
$KCl+K_2SO_4$	35.120 & 35.119
Mixed oxides	35.102
Free halogens	35.112
Organically combd I in pure dye	35.104
Organically combd Cl in pure dye	35.107
Pure dye	35.021(a)

Ext. D&C Red No. 7
(Alizarin Carmine)

	Method
Volatile matter (135°)	35.035
H_2O-insol. matter	35.037
$NaCl+Na_2SO_4$	35.115 & 35.119
Mixed oxides	35.102
Free acid as H_2SO_4	35.129
Pure dye	35.031

Ext. D&C Red No. 8
(Fast Red S, Fast Red A)

	Method
Volatile matter (135°)	35.035
H_2O-insol. matter	35.037
Ether exts	35.054
β-Naphthol	35.067(a)
$NaCl+Na_2SO_4$	35.115 & 35.119
Mixed oxides	35.102
Pure dye	35.020(c)

Ext. D&C Red No. 9
(Bordeaux Red)

	Method
Volatile matter (135°)	35.035
Ether exts	35.051
Chlorides & sulfates (as Ca salts)	35.115 & 35.119
$Fe_2O_3+Al_2O_3$	35.102
Pure dye	35.020(f)

Ext. D&C Red No. 10
(Azo Rubin Extra)

	Method
Volatile matter (135°)	35.035
H_2O-insol. matter	35.037
Ether exts	35.054
$NaCl+Na_2SO_4$	35.115 & 35.119
Mixed oxides	35.102
Pure dye	35.020(c)

Ext. D&C Red No. 11
(Fast Crimson GR)

	Method
Volatile matter (135°)	35.035
H_2O-insol. matter	35.037
Ether exts	35.054
Aniline	35.059
$NaCl+Na_2SO_4$	35.115 & 35.119
Mixed oxides	35.102
Pure dye	35.020(c)

Ext. D&C Red No. 12 (Royal Scarlet)	Method
Volatile matter (135°)	35.035
Ether exts	35.051
β-Naphthol	35.067(c)
$NaCl+Na_2SO_4$	35.115 & 35.119
$Fe_2O_3+Al_2O_3$	35.102
Pure dye	35.020(f)

Ext. D&C Red No. 13 (Croceine Scarlet MOO)	
Volatile matter (135°)	35.035
H_2O-insol. matter	35.037
Ether exts	35.054
Aniline	35.059
$NaCl+Na_2SO_4$	35.115 & 35.119
Mixed oxides	35.102
Pure dye	35.020(c)

Ext. D&C Red No. 14 (Oil Red XO)	
Volatile matter (100°)	35.035
Sulfated ash	35.101
H_2O-sol. matter	35.124
Matter insol. in CCl_4	35.039
Xylidine	35.059
β-Naphthol	35.067(b)
Pure dye	35.020(c)

Ext. D&C Violet No. 1 (Anthraquinone Violet)	
Volatile matter (135°)	35.035
H_2O-insol. matter	35.037
Ether exts	35.054
p-Toluidine	35.059
$NaCl+Na_2SO_4$	35.115 & 35.119
Mixed oxides	35.102
Pure dye	35.020(e)

Ext. D&C Violet No. 2 (Alizurol Purple)	
Volatile matter (135°)	35.035
H_2O-insol. matter	35.037
Ether exts	35.055
p-Toluidine	35.059
$NaCl+Na_2SO_4$	35.115 & 35.119
Mixed oxides	35.102
Pure dye	35.020(e)

Ext. D&C Yellow No. 1 (Metanil Yellow)	Method
Volatile matter (135°)	35.035
H_2O-insol. matter	35.037
$CHCl_3$-sol. matter	35.125
$NaCl+Na_2SO_4$	35.115 & 35.119
Mixed oxides	35.102
Pure dye	35.020(c)

Ext. D&C Yellow No. 2 (Metanil Yellow CA)	
Volatile matter (135°)	35.035
$CHCl_3$-sol. matter	35.125
Chlorides and sulfates (Ca salts)	35.115 & 35.119
$Fe_2O_3+Al_2O_3$	35.102
Pure dye	35.020(c)

Ext. D&C Yellow No. 3 (Fast Light Yellow)	
Volatile matter (135°)	35.035
H_2O-insol. matter	35.037
Ether exts	35.054
Aniline	35.059
$NaCl+Na_2SO_4$	35.115 & 35.119
Mixed oxides	35.102
Pure dye	35.020(c)

Ext. D&C Yellow No. 4 (Polar Yellow 5G)	
Volatile matter (135°)	35.035
H_2O-insol. matter	35.037
Ether exts	35.054
$NaCl+Na_2SO_4$	35.115 & 35.119
Mixed oxides	35.102
Pure dye	35.020(c)

Ext. D&C Yellow No. 5 (Fanchon Yellow, Hansa Yellow)	
Volatile matter (135°)	35.035
Sulfated ash	35.101
Matter insol. in toulene	35.044
2-Nitro-p-toluidine	35.060
Pure dye	35.031
M.p.	35.128

Ext. D&C Yellow No. 6 (DuPont Yellow)	Method
Volatile matter (135°)	35.035
H_2O-insol. matter	35.037
Ether exts	35.054
$NaCl+Na_2SO_4$	35.115 & 35.119
Mixed oxides	35.102
Pure dye	35.020(c)

Ext. D&C Yellow No. 7 (Naphthol Yellow S)	
Volatile matter (135°)	35.035
H_2O-insol. matter	35.037
Ether exts	35.054
$NaCl+Na_2SO_4$	35.115 & 35.119
Mixed oxides	35.102
Martius Yellow	35.080
Pure dye	35.020(c)

Ext. D&C Yellow No. 8 (Naphthol Yellow S Potassium Salt)	
Volatile matter (135°)	35.035
Ether exts	35.054
$KCl+K_2SO_4$	35.115 & 35.119
Mixed oxides	35.102
Martius Yellow	35.080
Pure dye	35.020(c)

Ext. D&C Yellow No. 9 (Yellow AB)	
Volatile matter (80°)	35.035
Sulfated ash	35.101
H_2O-sol. matter	35.124
Matter insol. in CCl_4	35.039
Interm diates	35.059
Pure dye	35.020(e)
M.p.	35.128

Ext. D&C Yellow No. 10 (Yellow OB)	
Volatile matter (80°)	35.035
Sulfated ash	35.101
H_2O-sol. matter	35.124
Matter insol. in CCl_4	35.039
Intermediates	35.059
Pure dye	35.020(e)
M.p.	35.128

Lakes	
Ether exts	35.051

35.017 Preparation of Sample—Official

Mix thoroly and weigh out portions required promptly. If weighing cannot be made directly into dish in which detn is to be made, use weighing bottles, placing in each bottle quantity approximating wt required, and weighing immediately.

Pure Dye

By Titration with Standard Titanous Chloride Soln—Official

35.018 APPARATUS—*See* FIG. 68

35.019 REAGENTS

(a) *Titanous chloride std soln.*—0.1*N*. *See* **42.040**.

(b) *Potassium dichromate std soln.*—*See* **42.022**.

(c) *Indicator.*—For many dyes $TiCl_3$ titrn end point is indicated by sharp decoloration. For some dyes change is so gradual that excess of $TiCl_3$ (not >0.3 ml ca 0.1*N* soln) is required, and suitable std soln of some other dye must be used for back-titrn (methylene blue serves well). In other cases it is better to use indicator that is reduced after original dye has reacted with the $TiCl_3$. Known quantity of FD&C Green No. 2 serves well for this purpose.

35.020 DETERMINATION

(**a**) Prep. 1.0% soln of sample in H_2O and place in 500 ml wide-mouth erlenmeyer quantity of soln equiv. to ca 20 ml 0.1*N* $TiCl_3$. Add 15 g Na citrate and H_2O to bring vol. to 150–200 ml. Heat to boiling and titr. with the std $TiCl_3$ soln.

(**b**) Prep. 0.5% soln of sample in alcohol. Proceed as in (**a**), substituting 50% alcohol for H_2O.

(**c**) Proceed as in (**a**), substituting 15 g Na acid tartrate for Na citrate.

(**d**) Proceed as in (**c**), using as indicator vol. FD&C Green No. 2 soln (freshly prepd) contg ca 10 mg dye. Det. $TiCl_3$ soln equiv. to vol. indicator soln used and deduct this quantity from total required for titrn.

(**e**) Prep. 0.5% soln of sample in alcohol. Proceed as in (**d**), substituting 50% alcohol for H_2O.

(**f**) (*18*) In wide-mouth erlenmeyer dissolve 0.2 g sample in 5 ml H_2SO_4, using stirring rod to break up any lumps, and mix well. Dil. with 100

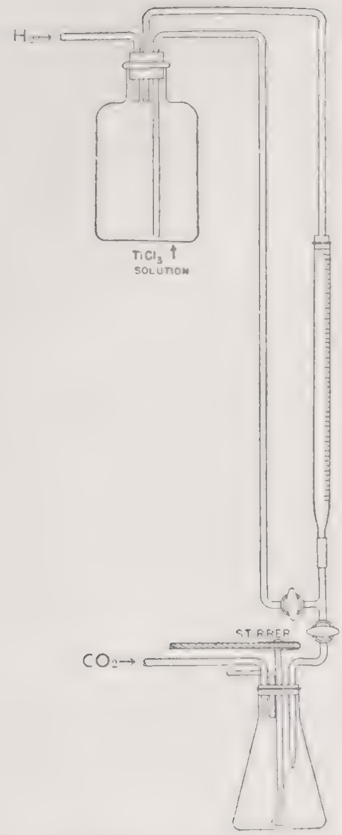

FIG. 68.—TITANOUS CHLORIDE TITRATION APPARATUS

ml alcohol and heat, with stirring, until all dye is in soln. Dissolve 20 g Na acid tartrate in 100 ml boiling H_2O and add 20 ml 30% NaOH soln. Stirring rapidly, add this soln to alc. dye soln. Titr. resulting soln with the std $TiCl_3$ soln.

(g) Place sample equiv. to ca 20 ml 0.1*N* $TiCl_3$ in 50 ml beaker, and pour down side of beaker 2 ml *fuming* H_2SO_4 (20% free SO_3). Stir well with glass rod and place on steam bath. After 30 min. pour sulfonated product into 500 ml wide-mouth erlenmeyer contg 100 g ice. Add few g cracked ice to material remaining in beaker and wash all color into flask. Add 50 ml alcohol and 20 g Na acid tartrate, heat, and titr. in usual manner.

(h) Proceed as in (g), but sulfonate at room temp.

35.021 *Gravimetrically—Official*

(a) Prep. 1.0% soln of sample in H_2O. Transfer 50 ml aliquot to 500 ml beaker, heat to boiling, add 25 ml HCl (1+49), and again bring to boil. Wash down sides of beaker with little H_2O, cover with watch glass, and keep on steam bath several hr or overnight. Cool to room temp., transfer ppt to weighed gooch with HCl (1+199), and wash with two 10–15 ml portions H_2O. Dry crucible and ppt 3 hr at 135°, cool in desiccator, and weigh. Calc. pure dye as follows:

$$\% \text{ pure dye} = \frac{\text{wt ppt} \times \text{conversion factor} \times 100}{\text{wt sample}}$$

(b) Prep. 1.0% soln of sample in ca 0.1*N* NaOH and proceed as in (a). No factor is required since colors for which this method is specified are not salts.

TABLE 2.—*$TiCl_3$ titration factors*

COLOR	MOLECULAR WEIGHT	G COLOR/ML 0.1*N* $TiCl_3$	ML 0.1*N* $TiCl_3$/G COLOR
FD&C Blue No. 1	792.8	0.03964	25.2
FD&C Blue No. 2	466.4	0.02332	42.9
FD&C Green No. 1	690.8	0.03454	29.0
FD&C Green No. 2	792.8	0.03964	25.2
FD&C Green No. 3	808.8	0.04044	24.7
FD&C Red No. 1	494.5	0.01236	80.9
FD&C Red No. 2	604.5	0.01511	66.2
FD&C Red No. 3	897.9	0.04490	22.3
FD&C Red No. 4	480.4	0.01201	83.3
FD&C Violet No. 1	733.9	0.03670	27.2
FD&C Yellow No. 5	534.4	0.01336	74.9
FD&C Yellow No. 6	452.4	0.01131	88.4
D&C Black No. 1	616.5	0.004404	227.1
D&C Blue No. 4	782.9	0.03915	25.5
D&C Blue No. 5	444.4	0.02222	45.0
D&C Blue No. 6	262.3	0.01311	76.3
D&C Blue No. 7	706.8	0.03534	28.3
D&C Blue No. 8	703.8	0.03519	28.4
D&C Brown No. 1	448.4	0.005605	178.4
D&C Green No. 4	786.9	0.03935	25.4
D&C Green No. 5	622.6	0.03113	32.1
D&C Green No. 6	418.5	0.02093	47.8
D&C Green No. 7	725.2	0.03626	27.6
D&C Orange No. 3	452.4	0.01131	88.4
D&C Orange No. 4	350.3	0.008758	114.2
D&C Red No. 5	480.4	0.01201	83.3
D&C Red No. 6	430.4	0.01076	92.9
D&C Red No. 7	424.4	0.01061	94.3
D&C Red No. 8	398.8	0.009970	100.3
D&C Red No. 9	444.5	0.01111	90.0
D&C Red No. 10	400.4	0.01001	99.9
D&C Red No. 11	397.4	0.009935	100.7
D&C Red No. 12	446.1	0.01115	89.7
D&C Red No. 13	421.2	0.01053	95.0
D&C Red No. 14	314.3	0.007858	127.3
D&C Red No. 15	360.0	0.009000	111.1
D&C Red No. 16	311.3	0.007783	128.5
D&C Red No. 17	352.4	0.004405	227.0
D&C Red No. 18	408.5	0.005106	195.8
D&C Red No. 19	479.0	0.02395	41.8
D&C Red No. 20	502.6	0.02513	39.8
D&C Red No. 30	393.3	0.01967	50.8
D&C Red No. 31	311.3	0.007783	128.5
D&C Red No. 33	467.4	0.01169	85.5
D&C Red No. 34	460.5	0.01151	86.9
D&C Red No. 36	327.7	0.003277	305.2
D&C Red No. 37	727.0	0.03635	27.5
D&C Red No. 39	329.3	0.008233	121.5
D&C Yellow No. 7	332.3	0.01662	60.2
D&C Yellow No. 8	376.3	0.01882	53.1
D&C Yellow No. 9	408.5	0.02043	48.9
Ext. D&C Black No. 1	731.7	0.009146	109.3
Ext. D&C Blue No. 1	319.8	0.01599	62.5
Ext. D&C Blue No. 2	776.0	0.01940	51.5
Ext. D&C Blue No. 3	690.8	0.03454	29.0
Ext. D&C Green No. 1	878.5	0.007321	136.6
Ext. D&C Orange No. 3	350.3	0.008758	114.2
Ext. D&C Orange No. 4	262.3	0.006558	152.5

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TABLE 2.—Continued

COLOR	MOLECULAR WEIGHT	G COLOR/ML 0.1N $TiCl_3$	ML 0.1N $TiCl_3$/G COLOR
Ext. D&C Red No. 1	566.5	0.01416	70.6
Ext. D&C Red No. 2	518.4	0.01296	77.2
Ext. D&C Red No. 3	634.6	0.03173	31.5
Ext. D&C Red No. 8	400.4	0.01001	99.9
Ext. D&C Red No. 9	397.4	0.009936	100.6
Ext. D&C Red No. 10	502.4	0.01256	79.6
Ext. D&C Red No. 11	509.4	0.01274	78.5
Ext. D&C Red No. 12	410.0	0.01025	97.6
Ext. D&C Red No. 13	556.5	0.006956	143.8
Ext. D&C Red No. 14	276.3	0.006908	144.8
Ext. D&C Violet No. 1	622.6	0.03113	32.1
Ext. D&C Violet No. 2	431.4	0.02157	46.4
Ext. D&C Yellow No. 1	375.4	0.009385	106.6
Ext. D&C Yellow No. 2	372.4	0.009310	107.4
Ext. D&C Yellow No. 3	380.4	0.009510	105.2
Ext. D&C Yellow No. 4	585.0	0.01462	68.4
Ext. D&C Yellow No. 5	340.3	0.003403	293.9
Ext. D&C Yellow No. 6	366.3	0.009158	109.2
Ext. D&C Yellow No. 7	358.2	0.002985	335.0
Ext. D&C Yellow No. 8	390.4	0.003253	307.4
Ext. D&C Yellow No. 9	247.3	0.006183	161.7
Ext. D&C Yellow No. 10	261.3	0.006533	153.1

TABLE 3.—*Conversion factors—precipitated color acids to specified salts*

COLOR	FACTOR
FD&C Red No. 3	1.074[a]
D&C Orange No. 6	1.090
D&C Orange No. 7	1.155
D&C Orange No. 9	1.110
D&C Orange No. 11	1.075
D&C Orange No. 12	1.130
D&C Orange No. 13	1.058
D&C Red No. 22	1.068
D&C Red No. 23	1.118
D&C Red No. 25	1.094
D&C Red No. 26	1.162
D&C Red No. 28	1.056
Ext. D&C Red No. 5	1.048
Ext. D&C Red No. 6	1.082

[a] Includes 1 molecule H_2O of crystn.

From Nitrogen Content—Official

35.022 APPARATUS—*See* **38.010**

35.023 REAGENTS

(a) *Hydriodic acid.*—To 9 vols 50% HI add 1 vol. 50% H_3PO_2 (clear soln should result).

(b) *Cigaret papers.*—Trim off glued edge and cut into 3 parts, each ca 23×40 mm.

(c) *Indicator.*—Dissolve 0.3 g Me red in 60 ml alcohol and dil. to 100 ml with H_2O. Dissolve 0.2 g methylene blue in 100 ml 50% alcohol and add this soln to Me red soln.

35.024 DETERMINATION

(a) *Colors requiring reduction prior to digestion.*—On tared cigaret paper, **35.023(b)**, accurately weigh quantity of sample expected to contain ca 2 mg N (10–25 mg). (Suitable aliquot of soln may be used if dye is H_2O-sol.) Place paper and sample in 30 ml Kjeldahl flask, add 0.5 ml of the HI mixt., and reflux gently 5–10 min., turning flask if necessary to insure soln and reduction. Distill off most of liquid, remove flask from heater, cool, add 5 ml H_2SO_4 (1+1), and evap. to fumes. (If considerable I remains, add 1–2 ml H_2O and again take to fumes.) Remove flask from heater, cool, wash down with 0.5 ml H_2O, and add 0.6 g Na_2SO_4 and 0.5 ml *20% $Hg(OAc)_2$* soln. Place flask on digester and heat 1 hr after clearing (2.5 hr for dyes having ring N; add more H_2SO_4 if needed). Remove flask, cool, and add 10–12 ml H_2O to dissolve salts.

Set elec. controller connected to steam generator of micro-Kjeldahl distg app. so that 20 ml will distill in ca 10 min. Transfer soln from digestion flask with ca 10 ml H_2O. Add 5–6 ml NaOH soln (1+1) and 3 ml 21% $Na_2S_2O_3$ soln.

Arrange 50 ml erlenmeyer contg 5 ml 2% H_3BO_3 soln and 3 drops of the indicator soln so that outlet from condenser dips below level of liquid. Steam distill 5 min. Lower receiving flask so that condenser outlet is above liquid in receiver and distill 1–2 min. to wash out condenser tube.

Titr. the NH_3 with std acid. 1 ml 0.02*N* acid = 0.28 mg N. Det. blank on all reagents used.

$$\text{mg N found} \times 100/\text{mg sample} = \%\ \text{N}.$$

$$\%\ \text{N} \times 100/\%\ \text{N calcd from formula of color} = \%\ \text{pure dye}.$$

(b) *Colors that do not require reduction prior to digestion.*—Weigh sample and transfer to digestion flask as in (a). Add 2 ml H_2SO_4 or 5 ml H_2SO_4 (1+1). Heat until thoroly charred, remove from heater, cool, and add 0.6 g Na_2SO_4 and 0.5 ml *20% $Hg(OAc)_2$* soln. Wash down any material adhering to side of flask with smallest possible vol. H_2O. Proceed as in (a).

From Sulfur Content

35.025 Fusion Method—Official

Place ca 0.2 g accurately weighed sample in Parr calorimetric bomb and mix with ca 14 g Na_2O_2. Add ca 1 g sugar and ca 0.1 g $KClO_3$ to aid in igniting mass. Close bomb and ignite. Cool, unscrew cover, and place cup contg melt in beaker. Cover beaker with watch glass and add H_2O until cup is completely covered. After mass dissolves, remove cup and wash thoroly, adding washings to soln.

Cautiously acidify soln with HCl. After heating to near boiling few min., filter, and wash filter with hot H_2O. Make filtrate neutral to litmus and add 1 ml HCl. Dil. to ca 400 ml and heat to boiling. Add slowly, with stirring, slight excess hot 2% $BaCl_2$ soln. Digest ppt 1–2 hr on steam bath, let settle, and filter thru tared gooch. Wash ppt by decantation with three 25 ml portions hot HCl (1+99) and then transfer to crucible, using

ca 250 ml hot HCl (1+99) in all. Finally wash with ca 15 ml hot H_2O and dry at 135° to constant wt.

% total S = 0.1373 × g $BaSO_4$ × 100/wt sample; Total S − inorg. S (calcd from Na_2SO_4 found) = org. S;

% org. S × 100/% S calcd from formula of color = % pure dye.

CAUTION: *Do not lean over bomb when mixing with sugar and stay at distance when igniting!*

Perchloric Acid Digestion Method (*19*)—First Action

35.026 REAGENTS

(a) *Nitric-perchloric acid mixture.*—Mix 2 vols 70% HNO_3 and 1 vol. 72% $HClO_4$.

(b) *Boric acid soln.*—Satd (ca 5 g H_3BO_3/100 ml H_2O).

(c) *Tetrahydroxyquinone indicator.*—May be obtained as "THQ Prepared Sulfate Indicator" from W. H. & L. D. Betz, Philadelphia, Pa.

(d) *Barium chloride std soln.*—0.02*M*. Dissolve 4.886 g $BaCl_2.2H_2O$ in 1 L H_2O. Stdze by pptn as $BaSO_4$.

35.027 DETERMINATION

CAUTION: Some substances react with explosive violence when digested with $HClO_4$. Although following method has been found to be safe with wide variety of compounds, make trial digestion of small sample before analyzing substance not previously investigated. See Definition of Terms and Explanatory Notes.

Weigh sample contg 2–6 mg S and transfer to bottom of 30 ml Kjeldahl flask. (Aliquot of aq. soln may be used.) Add 3–5 ml H_2O, 1 ml HCl (1+1), 3 ml of the HNO_3–$HClO_4$ mixt., and 2 or 3 glass beads. Place flask on digestion stand and boil gently until all HNO_3 is removed and boiling $HClO_4$ condenses ca ⅔ up neck of flask. If soln is not clear at this point, continue to heat until it clears and then heat 5–10 min. at b.p. of $HClO_4$.

Remove flask from digestion stand, cool, dil. mixt. to 8–10 ml with H_2O, and transfer to 200 ml erlenmeyer. Rinse digestion flask with three 5 ml portions H_2O and add to main soln. Add 3 drops phthln, and neutralize soln with NH_4OH. Place flask on hot plate and boil 2–3 min. after pink color is discharged. (Soln should be colorless; yellow or brown color indicates incomplete digestion.) Adjust vol. soln to 25 ± 5 ml by further boiling or by addn of H_2O.

Cool to room temp. and add ca 0.05*N* NH_4OH until soln is faint pink. Add 2 ml of the satd H_3BO_3 soln to discharge color. If color persists, add ca 0.05*N* $HClO_4$ until soln is colorless. Add 25 ml alcohol, 1 ml 10% NH_4Cl soln, and ca 0.2 g tetrahydroxyquinone indicator, and shake until indicator dissolves. Add the std $BaCl_2$ soln slowly until 1–2 ml from expected end point; then add 3 drops 10% $AgNO_3$ soln and continue titrn, adding the $BaCl_2$ soln dropwise. Agitate soln thoroly thruout titrn. End point is appearance of rose-pink throughout soln. (Change in color from yellow to pink is usually very sharp, and is readily detected after few practice titrns. It is preferable to carry out titrn in natural light, altho end point can be observed in strong artificial light.)

Conduct blank detn by digesting same quantities of reagents in same manner as in detn of sample. Wash digestion mixt. into flask contg known quantity of sulfate and continue as in regular detn. Ml $BaCl_2$ soln required in excess of calcd quantity is combined reagent and titrn blank. 1 ml 0.02*M* $BaCl_2$ = 0.642 mg S.

% total S = Net ml 0.02*M* $BaCl_2$ × 0.642 × 100/mg sample;

Total S − inorg. S = org. S;

% org. S × 100/% S calcd from formula of color = % pure dye.

By Spectrophotometric Measurement (20)—Official

35.028 APPARATUS

(a) *Spectrophotometer.*—Capable of accurate measurement of transmittance of solns in region 400–750 mμ; preferably with effective slit width of 10 mμ or less.

(b) *Two or more matched absorption cells.*

35.029 REAGENTS

(a) *Std sample of dye to be determined.*—Std samples should be carefully prepd and of highest attainable purity. Pure dye content of std samples must be accurately known if results are to be quant.

(b) *Solvents.*—Free from suspended matter.

35.030 STANDARDIZATION

Prep. series of solns of known concns of std sample and det. absorbance, *A* (optical density or log 1/transmittance) of solns, corrected for absorbance due to solvent and cell, at suitable wavelength. (Wavelength at which absorbance is max. is usually selected.) Adjust concns of solns to give absorbance values of 0.4–1.0 with instrument and cells used. Plot or tabulate data obtained.

35.031 DETERMINATION

Prep. sample soln in solvent used in stdzn. (Soln must be of such concn that absorbance obtained will be in range covered by stds examined.) Det. absorbance of this soln under same conditions used in stdzn.

Calc. "pure dye" content of sample from absorbance of sample soln and stdzn data as follows:

$$\text{Pure dye} = \frac{A_{\text{sample}}/\text{concn sample}}{A_{\text{std}}/\text{concn std}} \times \text{purity of std.}$$

If straight line does not result when absorbance and concn data obtained from examination of std soln are plotted, *i.e.*, if Beer's law does not hold, det. concn of "unknown" soln by comparison with data obtained from known soln of very nearly same concn.

35.032 Pure Dye in D&C Orange No. 15—Official

Reflux 0.1 g sample 45 min. with 25 ml HCl (1+1) and 25 ml alcohol. Cool, transfer to separator, and ext. with two 50 ml portions ether, then with 25 ml portions ether until no more color is extd. Wash ether exts with three 50 ml portions H_2O and filter thru small cotton plug in stem of funnel into weighed dish. Wash funnel and filter with little ether, adding washings to filtrate. Let ether evap., dry residue 30 min. at 100–105°, cool in desiccator, and weigh. Calc. % pure dye from wt extd D&C Orange No. 15.

Alizarin in Madder Lake (*21*)—Official

35.033 REAGENT

Sodium hydroxide-alcohol soln.—Mix 60 ml 10% NaOH soln with 45 ml alcohol and dil. to 300 ml with H_2O.

35.034 DETERMINATION

Weigh 0.2 g madder lake into 250 ml flask and add 25 ml HCl (1+1) and 25 ml alcohol. Reflux 45 min., cool, and wash mixt. into 250 ml separator with H_2O. Ext. with 50 ml portions ether until aq. layer is colorless. Wash combined ether solns with 20 ml portions of the NaOH-alcohol soln until ether layer is colorless. Combine alk. solns, acidify with few ml 6*N* H_2SO_4 (color changes from purple to orange), and re-ext. alizarin with six 25 ml portions ether. Combine ether exts and wash with 25 ml portions of the NaOH-alcohol soln until ether layer is colorless. Combine alk. washings and acidify with few ml H_2SO_4 (1+5). Re-ext. alizarin with six 25 ml portions ether, combine ether exts, wash 4 times with 25 ml H_2O, and discard washings. Filter ether soln thru qual. paper into tared 250 ml crystg dish. Wash paper with several 20 ml portions ether. Combine filtrate and washings and let ether evap. at room temp. Dry extd color in desiccator over H_2SO_4 to constant wt. Wt residue × 500 = % alizarin.

35.035 Volatile Matter—Official

Weigh accurately ca 2 g sample into tared weighed bottle ca 1.5″ diam., dry in air oven at temp. prescribed, **35.016**, 6 hr or overnight. Cool over efficient desiccant and reweigh. Report loss in wt as volatile matter.

INSOLUBLE MATTER

Water-Insoluble Matter—Official

35.036 APPARATUS

Prepared gooch crucible.—Digest good grade of retentive asbestos with HCl (1+3), wash free from acid, and decant to remove fine particles. Prep. well-packed asbestos mat of suitable thickness in gooch, wash with hot H_2O, dry, ignite, rewash, dry at 135°, cool in desiccator, and weigh. Repeat washing, heating, and drying to constant wt.

35.037 DETERMINATION

Dissolve 2 g sample in 200 ml hot H_2O and let soln cool to room temp. Filter thru tared gooch, **35.036**, wash with cold H_2O until washings are colorless, dry 3 hr at 135°, cool in desiccator, and weigh. Report increase in wt as H_2O-insol. matter.

35.038 Non-Volatile Water-Insoluble Matter—First Action

Ignite gooch contg total insol. matter, **35.037**, at 500° until all org. matter volatilizes. Cool in desiccator and weigh.

35.039 Carbon Tetrachloride-Insoluble Matter—Official

Mix 2 g sample with 100 ml CCl_4 in 250 ml beaker, stir, and heat to boiling. Filter hot soln thru weighed gooch, transfer residue in beaker to filter, and wash with 10 ml portions CCl_4 until washings are colorless. Dry 3 hr at 100–105° and weigh. Report increase in wt as matter insol. in CCl_4.

35.040 Toluene-Insoluble Matter—Official

Proceed as in **35.039** but use 1 g sample in 150 ml toluene.

35.041 Benzene-Insoluble Matter—Official

Proceed as in **35.039**, but substitute benzene for CCl_4.

Alkaline Dioxane-Insoluble Matter—First Action

35.042 REAGENT

Alkaline dioxane soln.—Mix 20 ml 10% NaOH soln and 80 ml H_2O with 300 ml dioxane.

35.043 DETERMINATION

Place 1 g sample in 250 ml beaker, add 200 ml of the alk. dioxane soln, and stir until sample dissolves completely (3–5 min.). Filter soln thru weighed gooch, wash remaining dye from beaker with small portion of the reagent, and then wash crucible with addnl 100 ml of the reagent. Finally, wash with 50 ml H_2O and dry 3 hr at 100–105°. Cool in desiccator and weigh. Increase in wt is matter insol. in alk. dioxane.

35.044 Toluene-Insoluble Matter— First Action

Place 1 g sample in weighed gooch and place weighed cotton pad on top of sample. Support crucible in Soxhlet extn app. so that bottom of crucible is slightly above top of siphon tube. Ext. with toluene until no more dye can be removed. Remove crucible, warm on steam bath until all toluene evaps, and dry 3 hr at 100–105°. Increase in wt minus wt cotton pad is toluene-insol. matter.

35.045 Acetone-Insoluble Matter— First Action

Proceed as in **35.044**, but substitute acetone for toluene.

35.046 Alcohol-Insoluble Matter— First Action

Proceed as in **35.044**, but substitute alcohol for toluene.

35.047 Ether-Insoluble Matter— First Action

Proceed as in **35.044**, but substitute ether for toluene.

35.048 Carbon Tetrachloride-Insoluble Matter—First Action

Proceed as in **35.044**, but substitute CCl_4 for toluene.

35.049 Xylene-Insoluble Matter— First Action

Proceed as in **35.044**, but substitute xylene for toluene.

35.050 Insoluble Matter (Alkaline Solution)—First Action

Proceed as in **35.037**, but use 1% NaOH soln or NH_4OH (1+14) instead of H_2O.

EXTRACTS

35.051 Isopropyl Ether Extract (*22*)— Official

Transfer 5 g sample to cellulose thimble and ext. with peroxide-free isopropyl ether, **35.053**, 2 hr in Soxhlet extn app. Pour ext. into weighed flat-bottom 100 ml dish, rinse extractor with 10 ml isopropyl ether, and drain into same dish. Let ether evap. and dry residue over H_2SO_4 to constant wt (±0.5 mg). Increase in wt represents isopropyl ether ext. plus small quantity of color.

To det. blank, including dissolved color, re-ext. original sample 2 addnl hr and subtract wt thus found from first ether ext. Difference is isopropyl ether ext.

35.052 Matter Extractable by Alcoholic Hydrochloric Acid—First Action

Place 2 g sample in 100 ml vol. flask and add 50 ml alcohol and 0.1 ml (2–3 drops) HCl. Shake ca 2 min. Dil. to mark with alcohol, mix, and filter. Place 50 ml filtrate in weighed dish, evap. to dryness on steam bath, dry at 98–100°, and weigh.

Ether Extracts—Official

By Extraction in Separator

35.053 REAGENT

Isopropyl ether.—Wash 1 L isopropyl ether with two 100 ml portions ca 0.5*N* NaOH, and then with three 100 ml portions H_2O.

35.054 DETERMINATION

(**a**) *Neutral ether extract*.—Place aq. soln contg 10 g sample in separator and dil. to 200 ml. Ext. with two 100 ml portions of the washed isopropyl ether, shaking 1 min. during each extn. Decant ether into clean separator and rinse first separator with 10 ml of the ether, decanting into second separator. Reserve aq. color soln for (**b**). Wash combined exts with 20 ml portions H_2O until washings are colorless. Decant ether into beaker, rinse separator with 10 ml isopropyl ether, and decant into same beaker. Place beaker on H_2O bath or steam bath in dust-free atmosphere, let ether evap. to 50 ml, and transfer to weighed flat-bottom 200 ml crystg dish previously dried to constant wt over efficient desiccant. Rinse beaker with 10 ml isopropyl ether and drain into same dish. Evap. remaining ether and dry to constant wt (±0.5 mg) in desiccator. Increase in wt = neutral ether ext.

CAUTION: Do not fill beaker or dish more than ⅓ full and do not let isopropyl ether boil.

(**b**) *Alkaline ether extract*.—To reserved aq. color soln, (**a**), add 2 ml 10% NaOH soln and proceed as in (**a**), except wash ether ext. with ca 0.1*N* NaOH instead of H_2O. Reserve aq. color soln for (**c**). Increase in wt = alk. ether ext.

(**c**) *Acid ether extract*.—To color soln reserved from (**b**), add 3 ml HCl (1+1) and proceed as in (**a**), except to wash ether with HCl (1+199)

instead of H_2O. Discard color soln. Increase in wt = acid ether ext.

35.055 *By Extraction in Continuous Extractor*

(a) *Neutral ether extract.*—Dissolve 5 g sample in vol. H_2O suitable for use in 250 ml continuous extractor and ext. with ca 100 ml isopropyl ether, **35.053**, 5 hr. Transfer ext. to separator, rinse flask with 10 ml of the ether, and add to main ext. Proceed as in **35.054(a)**, beginning "Wash combined exts . . ."

(b) *Alkaline ether extract.*—To aq. soln in extractor add 2 ml 10% NaOH soln and proceed as in (a), except wash ether with ca 0.1*N* NaOH instead of H_2O.

(c) *Acid ether extract.*—Add 3 ml HCl (1+1) to alk. aq. soln of sample in extractor and proceed as in (a), except wash ether with HCl (1+199) instead of H_2O.

35.056 Ether Extracts from Alkaline Solution—Official

Dissolve 5 g sample in vol. 0.5*N* NaOH suitable for use in 250 ml continuous extractor and proceed as in **35.055(b)**.

35.057 Petroleum Ether Extract (*22*)—Official

Transfer 5 g sample to cellulose thimble and ext. with petr. ether 1 hr in Soxhlet extn app. Transfer ext. to tared 100 ml crystg dish, rinse extractor with 10 ml petr. ether and drain into same dish, and let evap. spontaneously. Dry in desiccator overnight and weigh. Increase in wt = petr. ether ext.

INTERMEDIATES

Volatile Amines (*23*)—Official

35.058 REAGENTS

(a) *Sodium nitrite soln.*—Dissolve 79 g $NaNO_2$ in 1 L H_2O.

(b) *Sulfamic acid soln.*—Dissolve 97 g NH_2HSO_3 in 1 L H_2O.

(c) *Coupling soln.*—In 500 ml wide-mouth flask dissolve 0.1 g 1-(4-sulfophenyl)-3-methyl-5-pyrazolone in 2 ml ca 2.5*N* NaOH, and add 50 ml H_2O and 10 g Na citrate. Cool, and store at 10° or below until used. This reagent must be freshly prepd.

35.059 DETERMINATION

Place 10 g sample, 200 ml H_2O, and 5 g Na citrate in 500 ml round-bottom flask with ₸ neck. Connect flask to condenser with suitable connecting tube. Distill 100 ml into 100 ml graduated cylinder contg 1 ml HCl, regulating rate of distn to require ca 1 hr.

Place distillate in 500 ml erlenmeyer (washing out graduate into flask with few ml H_2O), cool in ice to <10°, and add 1 ml of the $NaNO_2$ soln. Mix well and let stand in ice bath 30 min. Then add 2 ml of the NH_2HSO_3 soln, mix well, and wash down sides of flask with few ml ice-H_2O. Let stand in ice 2–3 min.; then pour into flask contg the coupling soln. Let stand at least 1 hr, heat on steam bath 30 min., then heat to boiling, and titr. yellow color with 0.1*N* $TiCl_3$. If 1% soln of FD&C Green No. 2 is used as indicator, make correction for indicator blank. 1 ml 0.1*N* $TiCl_3$ = 2.3 mg aniline, 2.6 mg toluidine, 3.0 mg xylidine, or 3.3 mg pseudocumidine.

35.060 Non-Volatile Unsulfonated Amines (*24*)—Official

Place 10 g sample in Soxhlet extn thimble and ext. with petr. ether at least 4 hr or until ext. siphoning over is colorless. (Note: Use ether for detn of *p*-nitroaniline.) Transfer solvent to 500 ml separator, rinse extn flask with two 10 ml portions fresh solvent, and add washings to main ext. Wash combined exts with 30 ml H_2O and transfer ether layer to 500 ml wide-mouth erlenmeyer. Add 50 ml H_2O to ext. and evap. on steam bath with gentle air stream until all volatile solvent is driven off.

Remove flask from steam bath, cool to room temp., transfer contents to 110 ml vol. flask, and dil. to vol. with H_2O. Filter soln thru retentive paper and transfer 100 ml aliquot from filtrate to 500 ml wide-mouth erlenmeyer. Add ca 15 g Na tartrate to soln and heat to boiling. Titr. with std 0.1*N* $TiCl_3$ as in **35.020(a)** until yellow color disappears. End point is more readily detected when 1 ml std soln of FD&C Green No. 2 (Light Green SF Yellowish) is added as indicator near end of titrn.

One ml 0.1*N* $TiCl_3$ = 2.3 mg *p*-nitroaniline, 2.5 mg 3-nitro-4-aminotoluene or 2-nitro-4-aminotoluene, 2.8 mg 2-nitro-4-methoxyaniline, or 2.9 mg 2-chloro-4-nitroaniline.

Lake Red C Amine in D&C Red Nos. 8 and 9 (*25*)—Official

35.061 REAGENT

Std soln of Lake Red C Amine.—10 mg/L. Dry purified sample of Na salt of Lake Red C Amine 4 hr at 105°. Transfer 100 mg to 200 ml vol. flask and add ca 150 ml H_2O. When all amine dissolves, dil. to vol. with H_2O and mix well. Transfer 10 ml aliquot to 500 ml vol. flask, dil. to ca 450 ml with H_2O, and make slightly alk. (pH ca 8) with NH_4OH (1+1). Dil. to mark with H_2O and mix thoroly.

35.062 DETERMINATION

Transfer 1.0 g sample to 500 ml tall beaker. Wet sample with 5 ml acetone and then add 100

ml 2% $BaCl_2$ soln. Boil mixt. 10 min. and filter hot thru Whatman No. 12 folded paper into 500 ml Pyrex separator. Return paper and dye slurry to original beaker, repeat boiling H_2O extn, and filter as before. Make third hot H_2O extn in same manner. Discard paper and dye slurry.

Cool combined filtrates, acidify with 5 ml HCl (1+1), and ext. with three 20 ml portions benzene. Wash combined benzene exts with 20 ml portion H_2O and add wash H_2O to combined filtrates. Insert cotton plug into separator stem and filter soln into 500 ml beaker. Add boiling chips and boil 15–20 min. to remove benzene. Cool soln and adjust pH to ca 8.0 with NH_4OH (1+1). Transfer alk. soln to 500 ml vol. flask, dil. to mark with H_2O, and mix thoroly.

Det. absorbance of sample, A, and of std, A', at 247 mμ. Calc. % Lake Red C Amine in sample from following equation:

%Lake Red C Amine $= A \times 0.91 \times C' \times 100/A' \times W$, where C' is concn std (mg/L) and W is wt sample (mg).

β-Naphthol—Official

Method I.

35.063 REAGENT

Diazotized sulfanilic acid soln.—Approx. 0.05*N*. Dissolve 4.779 g sulfanilic acid in 500 ml H_2O to which has been added 5 ml HCl. Prep. ca 0.05*N* $NaNO_2$ by dissolving 1.04 g $NaNO_2$ in 300 ml H_2O. Place 40 ml of the sulfanilic acid soln in 100 ml vol. flask, cool to 5°, add 44 ml of the $NaNO_2$ soln, and let diazotize, testing for excess HNO_2 with starch-iodide paper. Destroy any excess with few mg sulfamic acid. Dil. to vol. with H_2O.

35.064 DETERMINATION

Weigh 10 g sample into 25×80 mm seamless extn thimble, place in Soxhlet extn app. of suitable size, and ext. with petr. ether (b.p. 35–60°) 8 hr. (Some dye extd along with β-naphthol may collect on sides of flask.)

Disconnect extractor, add 150 ml HCl (1+10) to extn flask, gently boil off ether on hot plate, and filter thru glass wool. Rinse flask several times with small quantities of the HCl, filtering each rinse thru glass wool, and dil. to 250 ml with H_2O in vol. flask. Mix soln thoroly and divide into 2 equal portions. Adjust pH of each portion to neutrality with dil. NaOH soln, using phthln. Reserve 1 soln for blank.

Add 10 g $NaOAc.3H_2O$ to other portion and cool to 5° in ice bath. Add slowly 25 ml of the diazotized sulfanilic acid soln, stir 5 min., and test for excess reagent with alk. β-naphthol soln on spot paper. If test is negative, add addnl reagent until positive test is obtained. Let stand 1 hr (or longer).

Heat on H_2O bath 30 min. to decompose excess reagent and test with β-naphthol soln on spot paper to det. that decomposition is complete. To each of coupled and uncoupled portions add 10 g Na bitartrate dissolved in 50 ml hot H_2O. Titr. uncoupled blank with 0.1*N* $TiCl_3$ to direct and colorless end point. Titr. coupled portion until dye reduces to yellow. Add 1–2 ml excess $TiCl_3$ soln and back-titr. *immediately* with stdzd methylene blue, or other suitable dye soln. (Back-titrn is necessary for coupled portion.)

Subtract ml required for blank from ml required for coupled portion, and calc. as % β-naphthol. 1 ml 0.1*N* $TiCl_3$ = 0.0036 g β-naphthol.

Method II.

35.065 APPARATUS

Spectrophotometer suitable for measurements at 490 mμ.—*See* **35.028**(a).

35.066 REAGENTS

(a) *1-(4-Nitrophenylazo)-2-hydroxynaphthalene std soln.*—Dissolve 0.100 g pure 1-(4-nitrophenylazo)-2-hydroxynaphthalene in 200 ml $CHCl_3$. Make suitable dilns with $CHCl_3$ to give solns contg 5 and 10 mg/L, resp.

(b) *Isopropyl ether.*—Wash once with 0.1*N* NaOH.

(c) *p-Nitrobenzenediazonium chloride soln.*—Dissolve 20 mg *p*-nitroaniline in 2 ml HCl and dil. to 200 ml with H_2O. Add 100 g crushed ice and stir until temp. of soln is 5–10°. Add 2 ml 10% $NaNO_2$ soln and stir 10–15 min. Then add small portions of 10% sulfamic acid soln until soln gives negative test with starch-iodide paper.

35.067 DETERMINATION

(a) *Colors soluble in H_2O.*—Dissolve 2.0 g sample in 250 ml H_2O. Make soln acid with 5 ml 6*N* HCl and ext. with six 30 ml portions isopropyl ether. Wash combined ether exts with 20 ml 0.1*N* HCl (discard) and ext. with six 30 ml portions 0.1*N* NaOH.

Cool combined alk. β-naphthol exts to 5–10° with crushed ice and add the *p*-nitrobenzenediazonium chloride soln slowly with constant stirring. Stir reaction mixt. 15 min., heat to 90° on steam bath, remove from steam bath, cool to room temp., and ext. with 20 ml portions $CHCl_3$ until $CHCl_3$ exts are colorless. Wash combined $CHCl_3$ exts with 30 ml 0.1*N* NaOH. Filter $CHCl_3$ soln thru cotton pledget into 500 ml vol. flask and dil. to vol. with $CHCl_3$. Det. absorbance of std, A', and of unknown, A, at 490 mμ.

$$\% \ \beta\text{-Naphthol} = (A/A') \times (\text{concn std soln in mg/L}) \times (144/293) \times (1/40).$$

(b) *Colors soluble in isopropyl ether.*—Dissolve 2.0 g sample in 250 ml isopropyl ether, warming

on steam bath to aid soln, and ext. with six 30 ml portions 0.1N NaOH. Wash combined alk. exts with 30 ml isopropyl ether and proceed as in (a), beginning "Cool combined alk. β-naphthol exts . . . "

(c) *Colors insoluble in H_2O or isopropyl ether.*—Ext. 10 g sample with isopropyl ether 8–10 hr in Soxhlet extn app. Transfer ether ext. to 1 L separator. Rinse extn flask with two 20 ml portions isopropyl ether and add to main ext. Ext. isopropyl ether with six 30 ml portions 0.1N NaOH and wash combined alk. exts with 30 ml isopropyl ether.

Dil. alk. soln to exactly 500 ml with 0.1N NaOH, place 100 ml aliquot in beaker, and proceed as in (a), beginning "Cool combined alk. β-naphthol exts . . . "

α-Naphthol in Ext. D&C Orange No. 3—Official

35.068 APPARATUS—*See* **35.028**(a)

35.069 REAGENT

4-(4-Nitrophenylazo)-1-hydroxynaphthalene std soln.—2 mg/L. Dissolve 10 mg 4-(4-nitrophenylazo)-1-hydroxynaphthalene in 100 ml warm acetone, cool, transfer to 500 ml vol. flask, and dil. to vol. with $CHCL_3$. Dil. 10 ml aliquot to 100 ml with $CHCL_3$.

35.070 DETERMINATION

Place 5 g Ext. D&C Orange No. 3 in cellulose extn thimble and ext. in Soxhlet extn app. with ether at least 4 hr. Transfer ext. to 500 ml separator, wash extn flask with two 10 ml portions ether, and add washings to main ext. Wash combined exts once with 20 ml H_2O and ext. with six 30 ml portions 0.1N NaOH. Dil. combined alk. exts to 500 ml with 0.1N NaOH.

Couple 100 ml aliquot of this soln with p-nitrobenzenediazonium chloride as in **35.067**(a). After coupling mixt. has been warmed on steam bath, make soln acid to litmus with 6N HCl, cool, and ext. with 20 ml portions $CHCL_3$ until aq. layer is colorless. Wash combined $CHCL_3$ exts with 20 ml H_2O, filter thru cotton pledget into 500 ml vol. flask, and dil. to vol. with $CHCl_3$. Det. absorbance of std, A', and of unknown, A, at 464 mμ.

$$\% \ \alpha\text{-Naphthol} = (A/A') \times (\text{concn std soln in mg/L}) \times (144/293) \times (1/20).$$

35.071 Pyrene in D&C Green No. 8—First Action

H_2O-insol. matter, **35.037,** contains all of pyrene as well as other H_2O-insol. material. Ext. this residue with 50 ml ether, filter into weighed dish, wash filter with ether, and add washings to filtrate. Evap. ether at 40–50°, dry over H_2SO_4 in desiccator 3 hr, and weigh. Increase in wt is pyrene.

Phthalic Acid Derivatives (*26*)—Official

(Applicable to FD&C Red No. 3; D&C Orange Nos. 5, 6, 7, 8, 9, 10, 11, 12, 13, and 17; D&C Red Nos. 21, 22, 23, 24, 25, and 26; D&C Yellow Nos. 7, 8, and 9; and Ext. D&C Orange No. 2)

35.072 REAGENTS

(a) *Ethyl acetate.*—Absolute, reagent grade.

(b) *Phthalic acid std soln.*—Weigh accurately 0.130–0.135 g K acid phthalate, dissolve in H_2O, and dil. to 500 ml. Dil. 10 ml of this soln to 200 ml with ca 0.1N HCl. Calc. concn phthalic acid (mg/100 ml) = mg $KHC_8H_4O_4 \times 0.00813$.

35.073 DETERMINATION

(a) *Water-soluble salts.*—Wash 2 g sample, accurately weighed, into 250 ml beaker with ca 100 ml H_2O. Heat nearly to boiling and add slowly, with stirring, HCl (1+9) until pptn appears complete. Add addnl 8.5 ml HCl (1+9), dil. to ca 150–160 ml, and digest on steam bath 1–2 hr. Cool to room temp., wash into 200 ml vol. flask, and dil. to vol. with H_2O. Filter thru dry paper.

Pipet 50 ml filtrate into 125 ml separator (use no grease on stopcocks) and ext. with 30 ml EtOAc. Transfer aq. phase to another separator and ext. with 25 ml EtOAc. Again transfer aq. phase to third separator and ext. with 20 ml solvent. Pass three successive 50 ml portions H_2O thru funnels in same order that extns were made. Discard EtOAc, combine aq. exts, and evap. to dryness. (It is convenient to reduce to small vol. on hot plate with aid of air jet and then evap. to dryness on steam bath.)

Dissolve residue in H_2O and transfer to 100 ml vol. flask. Add 8.5 ml HCl (1+9) and dil. to vol. Filter thru dry paper and det. absorbance of soln at 230, 262, and 276 mμ in spectrophotometer, **35.028**(a), against 0.1N HCl as blank. (If soln is too concd for accurate readings, dil. aliquot to more suitable concn with 0.1N HCl and multiply final result by diln factor.)

Measure absorbance of std phthalate soln at same wavelengths.

(b) *Color acids.*—To 2 g sample add 6 ml 10% NaOH and few ml H_2O, and mix until color dissolves. Dil. to ca 100 ml and proceed as in (a) beginning "Heat nearly to boiling . . . "

35.074 CALCULATIONS

Calc. quantity Y for both sample and std as follows:

$$Y = [A_{230} - (A_{230} - 0.7A_{276})] - A_{262};$$

then

$$\% \text{ phthalic acid} = (Y_{sample}/Y_{std}) \times (\text{concn std soln in mg/100 ml}) \times 0.2.$$

35.075 *Applicable to D&C Yellow No. 10*

Dissolve 1 g sample in H_2O and wash into continuous extractor. Add ca 1 ml HCl/100 ml soln and ext. 8 hr with ca 250 ml ether. Transfer ether to separator. Rinse extn flask with 2 small portions ether and add washings to main ext. Wash ether ext. with four 10 ml portions HCl (1+199). Combine washings in separator and ext. with 50 ml ether. Combine ether solns and ext. with four 10 ml portions 1% NaOH soln. Collect alk. exts in beaker and evap. to dryness. Dissolve residue in H_2O and transfer to 200 ml vol. flask. Add 2 ml HCl and dil. to vol. Proceed as in **35.073**(a), beginning "Filter thru dry paper . . ."

34.076 *Applicable to D&C Red No. 19 and D&C Yellow No. 11*

(a) *D&C Red No. 19.*—Weigh 0.5 g sample into beaker and dissolve in 20 ml hot H_2O. Cool to room temp. and transfer to 125 ml separator. Rinse beaker with 5 ml H_2O and add wash H_2O to separator. Add 80 ml $CHCl_3$ and 2 ml 10% NaOH soln, and shake vigorously 1 min. Drain $CHCl_3$ layer and wash aq. phase with two 30 ml portions $CHCl_3$, discarding $CHCl_3$. Add 7 ml HCl (1+9) and wash with two 30 ml portions $CHCl_3$, discarding $CHCl_3$. Transfer aq. soln to beaker, rinse funnel with 10 ml H_2O, and transfer to same beaker. Evap. to dryness on steam bath with aid of air jet. Proceed as in **35.073**(a), beginning "Dissolve residue in H_2O . . ."

(b) *D&C Yellow No. 11.*—Wash 0.5 g sample into 125 ml separator with 80 ml $CHCl_3$. Add 20 ml 1% NaOH soln and proceed as in (a), beginning "shake vigorously 1 min."

SUBSIDIARY AND LOWER SULFONATED DYES

Orange II in Ext. D&C Orange No. 3 (*27*)—Official

35.077 APPARATUS—*See* 35.028(a)

35.078 REAGENTS

Prep. following solns, using 0.1*N* NaOH as solvent:

(a) Soln contg 5–10 mg of sample/L.

(b) Soln contg 5–10 mg of FD&C Orange No. 1/L, recrystd twice from alcohol (1+1) and dried at 135°.

(c) Soln contg 10–20 mg of D&C Orange No. 4/L, recrystd twice from alcohol and dried at 135°.

(All concns should be accurately known.)

35.079 DETERMINATION

Det. absorbance of each soln at 455 and 515 mμ and calc. absorbance (mg/L) of the solns of the purified colors at each wavelength.

Calc. quantity of D&C Orange No. 4 present in sample from equations,

$$Ax + ay = A_{455} \quad \text{and} \quad Bx + by = A_{515},$$

where

A = Absorbance (mg/L) of soln of purified D&C Orange No. 4 at 455 mμ;
B = Absorbance (mg/L) of soln of purified D&C Orange No. 4 at 515 mμ;
a = Absorbance (mg/L) of soln of purified FD&C Orange No. 1 at 455 mμ;
b = Absorbance (mg/L) of soln of purified FD&C Orange No. 1 at 515 mμ;
x = Concn (mg/L) of D&C Orange No. 4 in sample;
y = Concn (mg/L) of FD&C Orange No. 1 in sample;
A_{455} = Absorbance of sample soln at 455 mμ; and
A_{515} = Absorbance of sample soln at 515 mμ.

$x \times 100$/concn soln (a) = % D&C Orange No. 4.

35.080 Martius Yellow—First Action

Dissolve 5 g sample in 150 ml H_2O, add 5 ml HCl, and shake vigorously in separator 1 min. with 50 ml petr. ether (sp. gr. 0.65). Sep. solns and ext. aq. liquid again with 25–30 ml of the solvent. Combine petr. ether portions, decant into clean separator, and wash with 25 ml portions 0.25*N* HCl until washings are colorless. Remove Martius Yellow by shaking with few portions of 5% NaOH soln. Neutralize alk. dye soln with *tartaric acid*, add 5 g Na tartrate, and titr. with std $TiCl_3$ soln, using as indicator ca 10 mg FD&C Green No. 2 from freshly prepd soln. Det. blank on tartrate, FD&C Green No. 2, and H_2O. 1 ml 0.1*N* $TiCl_3$ = 0.002134 g Martius Yellow.

Subsidiary Dyes in FD&C Yellow No. 5 (*28*)—Official

Lower Sulfonated Dyes

35.081 SPECTROPHOTOMETRIC STANDARD

Either (a) purified disodium salt of 3-carboxy-5-hydroxy-1-*p*-sulfophenyl-4-phenylazopyrazole or (b) purified FD&C Yellow No. 5 (recrystd twice from alcohol (1+1); dried at 135°) may be used as std.

Dissolve 100 mg of (a) or (b) in 1 L H_2O. Transfer 10 ml aliquot to 100 ml vol. flask, add ca 1 g NH_4OAc, and dil. to mark with H_2O.

35.082 DETERMINATION

Dissolve 200 mg sample in 100 ml H_2O, heating on steam bath if necessary. To 50 ml of this soln add 1 ml HCl and ext. lower sulfonated dye by shaking soln successively in 3 separators, each contg 50 ml amyl alcohol. Wash amyl alcohol exts by shaking successively with 50 ml portions $0.25N$ HCl, until washings are practically colorless, passing each acid portion thru funnels in same order as original amyl alcohol extn. Dil. amyl alcohol exts in each funnel with 1–2 vols petr. ether and remove lower sulfonated dye by washing with several 10–20 ml portions H_2O, passing each portion thru the 3 funnels in reverse order to that previously followed. Transfer extd color to 100 ml vol. flask, add ca 1 g solid NH_4OAc, dil. to vol. with H_2O, and measure absorbance of unknown, A, and of std, A', in spectrophotometer, **35.028**(a), at 434 mμ, using 1 cm cells.

Using std (**a**): % subsidiary $= A/A'$.
Using std (**b**): % subsidiary $= A/1.1A'$.

Subsidiary Dyes in FD&C Yellow No. 6

Lower Sulfonated Dyes (29)—First Action

35.083 REAGENT

D&C Orange No. 4 std soln.—Dissolve 0.100 g pure D&C Orange No. 4 in 500 ml H_2O. To 5 ml of this soln add 1–2 ml $2N$ NH_4OAc and dil. to 100 ml.

35.084 DETERMINATION

To soln of 0.2 g sample in 20 ml H_2O add 1 ml HCl and dil. to 50 ml. Ext. by shaking soln successively in 3 separators, each contg 50 ml amyl alcohol. Wash amyl alcohol exts successively with 50 ml portions 5% NaCl soln until washings are colorless. Dil. amyl alcohol in each separator with 100 ml portions petr. ether (sp. gr. 0.65) and remove lower sulfonated dye by washing with several 10 ml portions H_2O, passing each portion thru the 3 separators in reverse order to that previously followed.

Transfer combined aq. exts to 100 ml vol. flask, add 1 ml $2N$ NH_4OAc, dil. to vol. with H_2O, and measure absorbance of unknown, A, and std, A', at 485 mμ.

$$\% \text{ subsidiary dye} = 0.5A/A'.$$

Higher Sulfonated Dyes (30)—Official

35.085 PREPARATION OF STANDARD SOLUTIONS

Dissolve 17.3 g anhyd. sulfanilic acid and 5.5 g Na_2CO_3 in 100 ml H_2O. Add 25 ml HCl, cool to 10° or below, and then add slowly, with stirring, 35 ml 20% $NaNO_2$ soln. Stir 10 min. and then destroy excess nitrite with sulfamic acid.

(**a**) *R-salt std.*—Couple diazonium compound suspension obtained above with suspension of 34.8 g R-salt in 150 ml aq. soln contg 10 g NaOH and 10 g Na_2CO_3. Filter off product, crystallize from aq. alcohol (1+1), and dry at 135°.

(**b**) *G-salt std.*—Proceed as in (**a**) but use 34.8 g G-salt instead of R-salt.

Det. pure dye content of each product as in **35.020**(a). 1 ml $0.1N$ $TiCl_3 = 0.01386$ g pure dye. Prep. std solns in HCl (1+25) contg 4.0 mg pure dye/L.

35.086 DETERMINATION

Dissolve 100 mg sample in 100 ml HCl (1+25). Dil. 10 ml of this soln with 40 ml HCl (1+25) and ext. by shaking soln successively thru 5 separators, each contg 50 ml amyl alcohol. Transfer acid layer to 100 ml vol. flask. Wash amyl alcohol exts with two 25 ml portions HCl (1+25), passing each portion thru separators in same order as used for original extn. Add washings to acid soln of subsidiary and dil. to 100 ml with H_2O.

(**a**) *R-salt isomer.*—Det. absorbance, A, of extd soln at max. (ca 490 mμ) in 5 cm cell. Det. absorbance (mg/L) of std soln, A', **35.085**(**a**), at same wavelength and in same cell.

(**b**) *G-salt isomer.*—Proceed as in (**a**), using std soln, **35.085**(**b**), but det. absorbance at 476 mμ.

(**c**) *Mixtures of G- and R-salt isomers.*—Proceed as in (**a**), using std soln, **35.085**(**a**). Det. absorbance at 476 mμ where both isomers have ca same absorptivity.

$$\% \text{ subsidiary} = A/A'.$$

Subsidiary Dyes in D&C Red Nos. 6 and 7—First Action

35.087 REAGENTS

(**a**) *Potassium bromide-bromate soln.*—$0.05N$. Dissolve 1.3920 g $KBrO_3$ and 10 g KBr in 1 L H_2O.

(**b**) *Sodium thiosulfate std soln.*—Dissolve 12.5 g $Na_2S_2O_3.5H_2O$ in 1 L H_2O and stdze against the KBr-$KBrO_3$ soln as follows: Place 100 ml H_2O, 25 ml HCl, and 100 g crushed ice in 500 ml I flask. Add ca 20 ml of the KBr-$KBrO_3$ soln from buret as rapidly as possible. Stopper flask, and let stand in ice bath 10 min. Continue as in **35.088**, beginning "Add 2–3 g KI, . . ." Calc. value of the $Na_2S_2O_3$ soln in terms of the KBr-$KBrO_3$ soln.

35.088 DETERMINATION

Weigh 2.0 g sample into 1 L round-bottom flask of distn app., and add few anti-bumping pellets, 100 ml 10% NaOH soln, 25 ml *Me Cellosolve*, and 10 g $Na_2S_2O_4$. Attach adapter to condenser, leading tip below surface of 25 ml HCl (1+4), and heat mixt. to effect simultaneous reduction and distn.

When ca 50 ml distillate collects, let 150 ml H_2O drip into heated flask at rate ca equal that of distn. Continue distn until 300 ml collects in receiving vessel. Transfer liquid to 500 ml I flask, and conc. to 100 ml. Cool, and add 20 ml HCl and 100 g crushed ice.

Add the $KBr-KBrO_3$ soln to iced concentrate from buret until soln remains yellow at least 30 sec. Then add ca 5 ml more. Stopper flask and let stand in ice bath 10 min. Add 2–3 g KI, and titr. immediately with the $Na_2S_2O_3$ soln, using 0.5% starch soln as internal indicator near end point. 1 ml $0.05N$ $KBr-KBrO_3$ = 4.10 mg subsidiary dye.

Subsidiary Dyes in D&C Red No. 35 —First Action

Spectrophotometric Method (*31*)

35.089 REAGENTS

(**a**) *Chloroform.*—Redistd.

(**b**) *Alcohol.*—Special denatured No. 1 is satisfactory.

(**c**) *4-Toluene-azo-2-naphthol.*—Recrystd from alcohol. Dissolve 10 mg in alcohol and dil. to 1 L.

(**d**) *D&C Red No. 35.*—Recrystd from $CHCl_3$.

35.090 DETERMINATION

Ext. 2 g sample with $CHCl_3$ in Soxhlet app. until leachings are colorless. Cool, filter, and wash residue (D&C Red No. 35) and paper thoroly with alcohol. Evap. $CHCl_3$-alcohol filtrate to dryness (avoid spattering), and heat residue with 25 ml alcohol. Cool, transfer to 50 ml vol. flask, and dil. to vol. Remove insol. matter by filtration and dil. 5 ml aliquot to 250 ml with alcohol. Read absorbance, A, at 486 mμ. Repeat procedure, substituting 2 g of the D&C Red No. 35. Also det. absorbance, A', of the std 4-toluene-azo-2-naphthol soln.

$$\text{Total mg subsidiary dye} = 25A/A'.$$

(Total mg subsidiary dye − mg apparent subsidiary dye in D&C Red No. 35) $\times 1/20$ = % subsidiary dye.

Uranine (D&C Yellow No. 8) in Eosine (D&C Red No. 22)—First Action

35.091 APPARATUS

Adsorption column.—Glass tube 1200 mm long $\times$ 22 mm i.d., closed at both ends by 1-hole rubber stoppers. Thru upper stopper insert short-stem funnel, or other reservoir, and thru lower end insert glass tube connected to rubber tube, bearing pinch clamp, connected to second glass tube.

35.092 REAGENTS

(**a**) *Cellulose powder.*—Solka-Floc, SW-40-A (Brown and Co., Boston, Mass.), or equiv.

(**b**) *Uranine std soln.*—Dissolve 5 mg dye, 100% basis, in H_2O, add 2.0 ml 10% NaOH, and dil. to 1 L with 5% Na_2SO_4.

35.093 PREPARATION OF COLUMN

Slurry 20 g Solka-Floc in 800 ml H_2O and let stand 10 min. with occasional stirring. Place absorbent cotton pledget in tube, wet, and ram against rubber stopper at outlet. Pour cellulose slurry into tube, washing residual material into tube. Close outlet tube when liquid level drops to 5 mm above adsorbent. Replace the H_2O in column with 50 ml 5% Na_2SO_4 soln, stopping flow when liquid level falls to 5 mm above adsorbent.

35.094 DETERMINATION

Pipet 5 ml aliquot of 0.3% aq. eosine sample soln into beaker, add 0.1 ml 10% NaOH and 1 g anhyd. Na_2SO_4, and dil. to 20 ml with H_2O. Add 0.5 g cellulose powder, mix, and transfer to column. Wash residual dye into column with small portions 5% Na_2SO_4 soln, letting liquid level drop to 5 mm above adsorbent each time, until color is transferred; then raise eluate head to 1–2″.

Elute uranine with 5% Na_2SO_4 soln, using funnel reservoir or equiv. Discard eluate until visible uranine zone reaches outlet tube; then collect this subsidiary dye in 50 ml vol. flask. Add 0.1 ml 10% NaOH and dil. to mark with 5% Na_2SO_4. Det. absorbance at 490 mμ of this soln, A, and of the std uranine soln, A'. % Uranine = $A \times 1.67/A'$.

METALS

Lead—Official

Method I. (*32*)

(Applicable to colors not contg Ca, Ba, or Sr)

35.095 REAGENTS

(**a**) *Lead std soln.*—*See* **24.039**(**a**).

(**b**) *Dilute nitric acid.*—1%. *See* **24.039**(**b**).

(**c**) *Citric acid soln.*—50%. Special grade—low in Pb. *See* **24.039**(**d**).

(**d**) *Diphenylthiocarbazone* (*dithizone*) *soln.*—Stock soln of purified dithizone in $CHCl_3$ contg 100 mg/ml. Also working soln contg 20 mg/L. *See* **24.039**(**e**).

(**e**) *"Stripping" reagent.*—*See* **24.039**(**f**).

(**f**) *Potassium iodide soln.*—2%. *See* **24.039**(**g**).

(**g**) *Starch soln.*—0.5%. Weigh 1 g sol. starch. Make into thin paste with several ml cold H_2O, pour into 200 ml hot H_2O, and while still hot add 2–3 small crystals HgI_2 as preservative.

(**h**) *Sodium thiosulfate soln.*—Make stock $0.1N$ $Na_2S_2O_3$. Use 5 ml isoamyl alcohol/L as preservative. Prep. fresh $0.001N$ diln daily and stdze against std Pb soln.

(**i**) *Potassium cyanide soln.*—10%. Dissolve 50 g phosphate-free KCN in H_2O and dil. to 500 ml.

(**j**) *Hydroxylamine hydrochloride soln.*—10%.

Dissolve 10 g $NH_2OH.HCl$ in 20 ml H_2O and make slightly alk. with NH_4OH. Ext. Pb with dithizone. Remove excess dithizone with $CHCl_3$ and boil off any $CHCl_3$ remaining in aq. phase. Acidify with HCl and dil. to 100 ml.

(**k**) *Thymol blue indicator.*—0.1%. Dissolve 0.1 g thymol blue in H_2O, add enough 0.1*N* NaOH to change dye to blue, and dil. to 100 ml.

35.096 DETERMINATION

Transfer 5.00 g sample to 500 ml Kjeldahl flask, add 10 ml H_2SO_4 and 10 ml HNO_3, and heat. When SO_3 fumes begin to evolve, add 5 ml HNO_3 and heat until SO_3 again evolves. Repeat addn of HNO_3 each time SO_3 fumes appear until dye is completely in soln and digest is yellow. Then add 10 ml of mixt. of HNO_3 and 60–70% $HClO_4$ (1+1), and continue heating until digest is colorless or pale yellow and bulk of the H_2SO_4 is evapd.

Cool flask under running H_2O and neutralize soln by addns of small portions of NH_4OH. Add 20 ml of the citric acid soln and adjust to pH 8.5–9 with NH_4OH, using 4 drops of the thymol blue indicator. Add 5 ml 10% KCN soln.

Transfer alk. soln to 250 ml Pyrex separator. Ext. Pb with 20 ml portion of the dithizone soln contg 20 mg/L. (NOTE: If there is enough Fe present to cause excessive oxidation of the dithizone as indicated by yellow color in $CHCl_3$ layer, add 10 ml 10% $NH_2OH.HCl$ soln to reduce the Fe.) Let $CHCl_3$ layer settle and drain into another separator. Wash down floating globules of $CHCl_3$ with two 5 ml portions of less concd dithizone soln (4 mg/L) and add to receiving separator. Repeat extns with the more concd dithizone soln until no more of the red Pb dithizonate is observed. Make 2 more extns with 10 ml portions of the less concd dithizone soln.

Wash $CHCl_3$ ext. with 25–30 ml H_2O contg 1 drop NH_4OH. Drain washed $CHCl_3$ layer cleanly into third separator. Add 110 ml of the 1% HNO_3 and shake 1 min. Drain and discard $CHCl_3$ and ca 1 ml acid layer. Insert cotton plug into stem of separator to filter acid layer as it is withdrawn. Discard first 3 ml filtrate. Electrolyze 100 ml aliquot of filtrate as in **24.044**.

Method II. (33)

(Applicable to Al lakes)

35.097 APPARATUS

See Fig. 69. Pptn tube *B* is fitted with inlets for addn of sample and of H_2S and for release of H_2S and transfer of pptd PbS to filter in *C* (fine porosity fritted glass covered with Celite or other similar filter-aid). For 20–300 mmg Pb, ca 0.5 g filter-aid is enough to allow rapid filtration with complete retention of ppt. Filter must be thoroly

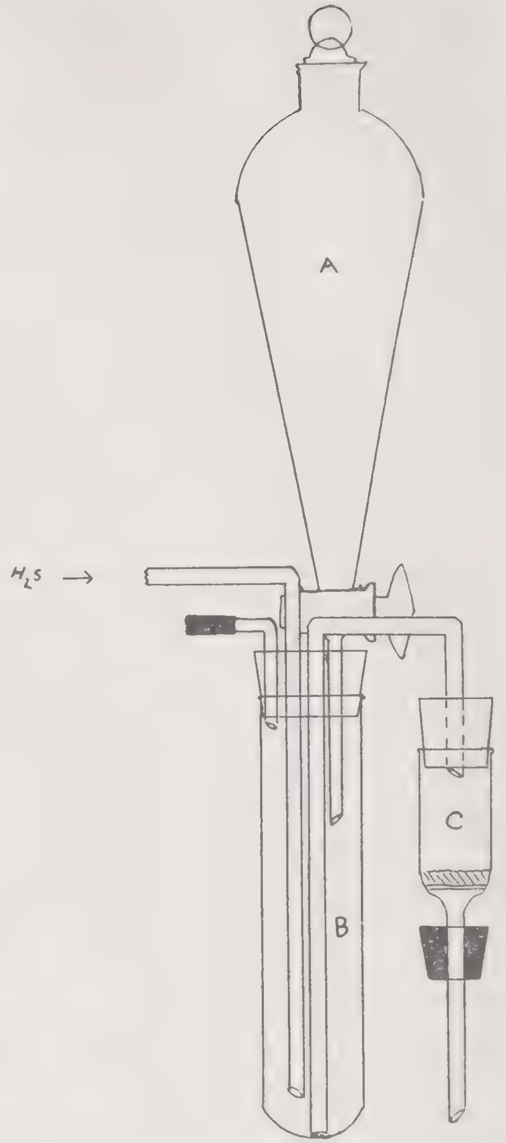

FIG. 69.—SULFIDING APPARATUS (FULL SCALE)

washed with HNO_3 followed by H_2O before use.

Wash all glassware successively with scouring powder, H_2O, HNO_3, and again with H_2O. Wash pptn app. with HNO_3 and H_2O between detns.

35.098 REAGENTS

All reagents should be Pb-free. *See* **24.039.** Any source of H_2S may be used. Scrub gas first with H_2SO_4 (1+1), and then with H_2O before passing into soln.

35.099 DETERMINATION

Weigh 2 g sample into 500 ml Kjeldahl flask, add 10 ml H_2SO_4 and 10 ml HNO_3, and digest over low flame until SO_3 fumes appear. Add 5 ml portions HNO_3 (waiting until SO_3 fumes appear before adding each succeeding portion) until all org. matter is in soln. Slowly add 5–10 ml mixt. of HNO_3 and 60–70% $HClO_4$ (1+1), and continue

digestion until white ppt formed shows first signs of spattering. Let flask cool, and cautiously add 5 ml H_2O and then few drops NH_4OH. Swirl flask vigorously and cool under running H_2O. Add 20 ml citric acid soln, **35.095(c)**, and adjust to pH 3.0–3.4 (bromophenol blue) with NH_4OH. Add 1 ml $CuSO_4$ soln (1 mg Cu/ml) and transfer soln to pptn tube, *B*, of sulfiding app., Fig. 69. Bubble H_2S thru soln 3–5 min. at rate of ca 2 bubbles/sec. and filter resulting suspension thru *C* at rate of ca.1 drop/sec. When filtration is complete, remove receiver contg filtrate and attach suction test tube.

Add 3 ml hot HNO_3 thru separator *A* and draw thru filter, followed by 2 ml hot H_2O. Detach filter and pass addnl 3 ml hot HNO_3 thru filter, wetting all sides. Again follow with 2 ml hot H_2O. If filter is still colored with PbS, wash again with hot HNO_3 and H_2O. Wash dissolved sulfides into pptn tube *B*, wetting all sides to take up any residual PbS and then into 50–100 ml g-s. erlenmeyer. Stopper, and shake few sec.; then remove stopper and boil until soln clears, to remove last traces of H_2S and to coagulate any free S present.

Transfer soln to 250 ml separator. Wash flask with two 5 ml portions H_2O and add washings to main soln. Add 10 ml of the citric acid soln, 5 ml 10% KCN soln, and few drops of the $NH_2OH.HCl$ soln, **35.095(j)**, to prevent oxidation of the dithizone; adjust pH to 8.5–9.5 (thymol blue) with NH_4OH and proceed with dithizone extn and electrolysis as in **24.042–24.044.**

35.100 *Method III. (33)*

(Applicable to Ca, Ba, and Sr lakes)

Place 2 g sample, 4 g Na_2CO_3, 6 g K_2CO_3, and 0.5 g $NaNO_3$ in Pt crucible and mix thoroly. Heat carefully until sample is carbonized; then heat to ca 850° and hold at that temp. 15 min. (If temp.-controlled furnace is available, it is only necessary to place fusion mixt. in the cold furnace and raise temp. gradually to 850° over 2 hr period. Usually 15 min. heating at 850° is enough to complete fusion.)

Let crucible and contents cool to $<100°$; then add 2 or 3 ml H_2O and heat over low flame, using care to prevent spattering, until contents can be sepd from crucible. Transfer fused mixt. to 150 ml beaker with aid of ca 25 ml hot H_2O. Boil until caked material is completely disintegrated, and filter thru retentive paper. Wash residue on filter with two 15 ml portions hot 5% Na_2CO_3 soln. Pb will be in both filtrate and residue. Transfer filtrate to separator and ext. Pb from filtrate as in **35.099.**

Dissolve residue on filter in 10–20 ml HCl (2+5), wash filter with H_2O, and add washings to the soln. Boil soln to expel CO_2; then transfer to separator and ext. Pb as above. Combine with $CHCl_3$ exts from sol. portion of fusion products and det. total Pb by electrolytic method, **24.042–24.044.**

35.101 **Sulfated Ash—Official**

Weigh accurately ca 5 g sample in weighing bottle and transfer to Pyrex Kjeldahl flask or tall beaker, washing out weighing bottle with little H_2O. Destroy org. matter by digestion, using 15 ml H_2SO_4 and adding HNO_3 as required. As bulk of HNO_3 is driven off, lower flame to avoid reaction on glass.

Transfer mixt. to weighed Pt dish and heat carefully over ring burner to avoid spattering. At first use low flame at safe distance below dish; then increase flame, and gradually bring it closer to dish. Continue heating until acid fumes decrease. If C remains, remove flame, let mass cool, and add H_2SO_4 dropwise until mass is moistened. Repeat treatment until all C is burned off and ash is white or reddish. Heat carefully with blast lamp until fusion takes place with production of clear liquid free from bubbles. Cool in desiccator and weigh. After deducting wt Na_2SO_4 equiv. to inorg. Na salts (chlorides, sulfates, carbonates, etc.) found in other detns, calc. % Na combined in dye.

35.102 **Mixed Oxides (Fe, Al, Ca, and Mg)—Official**

Moisten sulfated ash obtained in **35.101** with 2–3 ml HCl and evap. to dryness on steam bath. Warm residue with 20 ml HCl (1+19) until all sol. material dissolves, transfer to 100 ml vol. flask, dil. to 100 ml with H_2O, mix, and filter thru dry paper.

To 40 ml aliquot filtrate add 5 g NH_4Cl and neutralize with NH_4OH (1+1), boiling to drive off any excess. If ppt is very slight, disregard it; otherwise filter thru quant. paper, wash with H_2O contg trace of NH_4OH (reserving filtrate and washings), and ignite paper and ppt in weighed crucible. Weigh mixt. of Fe_2O_3 and Al_2O_3.

Place mixed oxides in 500 ml erlenmeyer and dissolve in aqua regia, boiling to drive off Cl. Add H_2O to ca 75 ml and add NH_4OH to incipient pptn. Dissolve ppt with as little HCl as possible, cool, and titr. ferric Fe present with 0.1*N* $TiCl_3$, **42.040,** using 5 g NH_4CNS as indicator. Calc. Fe as Fe_2O_3. To calc. Al_2O_3, deduct wt Fe_2O_3 from total wt mixed oxides. From wt Al_2O_3 calc. % Al.

Pass washed stream of H_2S into alk. filtrate from the Fe- and Al-hydroxides. White ppt indicates presence of Zn.

To second 40 ml aliquot filtrate add 250 ml H_2O to insure low concn of Mg, if present. Heat to boiling and add 3.5 g NH_4Cl and enough NH_4OH soln (1+99) to make soln barely alk.

Filter off pptd hydroxides of Fe and Al. Wash and discard ppt. Heat combined filtrate and washings to boiling and add 1 g NH_4 oxalate. Let cool and stand 1 hr, filter thru asbestos mat prepd on small Witt plate in glass funnel, and wash with very little H_2O, reserving combined filtrate and washings. Place mat in beaker, add 100 ml H_2O and 2 ml H_2SO_4, heat gently until CaC_2O_4 dissolves, and titr. with 0.1*N* $KMnO_4$. Calc. as Ca.

Heat reserved filtrate and washings to boiling and add 1*N* $NaNH_4HPO_4$ soln until there is no further pptn. While stirring add ca $\frac{1}{3}$ the vol. of NH_4OH soln (1+9). Let stand 3 hr, filter thru ashless paper, and wash with NH_4OH soln (1+49). Ignite filter and ppt in weighed crucible, cool in desiccator, and weigh the $Mg_2P_2O_7$. Calc. as Mg.

HALOGENS IN PURE COLORS

Iodine (*34*)—Official

35.103 REAGENTS

(a) *Saturated potassium permanganate soln.*—Dissolve 7 g $KMnO_4$ in 100 ml H_2O.

(b) *Sodium thiosulfate std soln.*—0.1*N* or 0.05*N*, depending on size of sample taken. Stdze against pure KIO_3.

35.104 DETERMINATION

Place accurately weighed sample contg ca 50 mg I in 500 ml tall beaker. Dissolve sample in ca 2 ml 30% NaOH soln, dil. to 100 ml, and add few glass beads and 15 ml satd $KMnO_4$ soln. Cover with watch glass, boil 5 min., and remove from heat. When boiling ceases, add carefully 10 ml HNO_3 and boil 5 min. more.

Remove beaker from heat and wash down cover glass and sides (excess $KMnO_4$ must be present). Add 5 ml 10% $NaNO_2$ soln quickly with swirling. ($KMnO_4$ color is destroyed, and brown suspension of MnO_2 is left.) Continue addn of the $NaNO_2$ soln dropwise until suspension begins to clear; then add the $NaNO_2$ soln cautiously, letting each drop react before next is added. When soln appears colorless by transmitted light, but some particles of solid MnO_2 remain, do not attempt to destroy these, but immediately add 1% $KMnO_4$ soln in 1 ml portions until soln becomes pink.

NOTE: If >2 ml is required or if brown color appears, add at once 10 ml of the dil. $KMnO_4$ soln and again heat to boiling. Repeat dropwise addn of the $NaNO_2$ soln and again add the dil. $KMnO_4$ soln to pink color.

Filter soln rapidly with suction thru medium porosity fritted glass filter into wide-mouth 500 ml flask. Wash beaker and filter thoroly with H_2O. (Soln must remain pink after filtration.) Add the $NaNO_2$ soln dropwise with shaking until 1 drop has been added in excess of that required to decolorize soln. Add 5 ml *10% sulfamic acid soln*, wash down sides of flask, and swirl contents. Cool soln to room temp., add 2–3 g solid KI, and titr. liberated I with the std $Na_2S_2O_3$ soln, using starch indicator, **35.095**(g). Det. blank on reagents used. 1 ml 0.1*N* $Na_2S_2O_3$ = 0.002115 g I.

Total I − inorg. I = organically combined I.

Bromine (*35*)—Official

35.105 APPARATUS (Fig. 70)

Consists of 100 ml round-bottom flask with 24/40 ₸ inner joint; condenser with jacket ca 130 mm long; and absorption flask with 2 bulbs. Condenser is equipped with 12/30 and 24/40 ₸ inner joints. Small dropping funnel is fused to tube above jacket. Absorption flask has outer 12/30 ₸ joint. Small springs (not shown) are attached to hooks on joints to keep app. tightly connected during use.

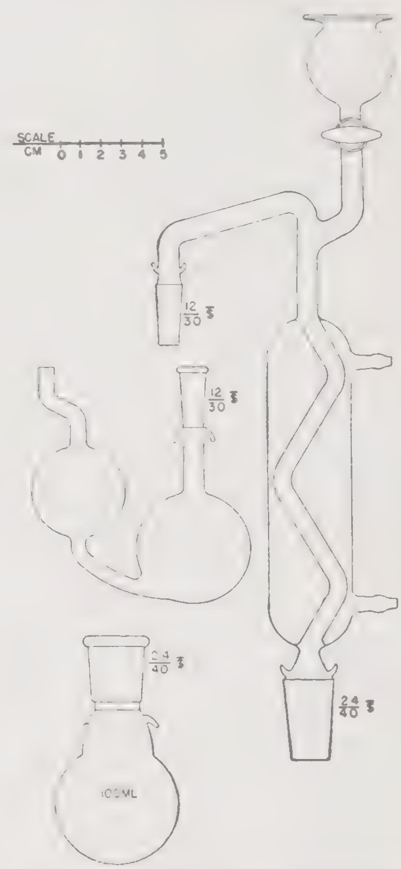

FIG. 70.—BROMINE APPARATUS

35.106 DETERMINATION

Place sample calcd to contain 40–60 mg Br in oxidation flask and dissolve in 2 ml 10% NaOH soln and 8 ml H_2O, or use 10 ml aliquot of suitable soln of dye. Lubricate joints of app. with H_3PO_4 and connect flask to condenser. Place ca 15 ml

1% $N_2H_4.H_2SO_4$ *soln* and 5 ml 10% NaOH soln in absorption flask and connect to app.

Add 5 ml CrO_3 soln (1+1) thru addn tube, wash down with 2–3 ml H_2O, and then slowly add 10 ml H_2SO_4. If vigorous reaction begins, let it subside before heating flask; if reaction does not begin as acid is added, heat gently with small flame, but remove flame before reaction becomes too vigorous, otherwise reaction mixt. may foam up into condenser. When reaction subsides and most of dye is in soln, heat mixt. to boiling. When foaming subsides, add 5 ml H_2SO_4 thru dropping funnel, boil 10 min., add another 5 ml H_2SO_4, and boil again 10 min. Drain H_2O from condenser and boil reaction mixt. until 2–3 drops H_2O distill into absorber.

Disconnect absorption flask, wash contents into 500 ml I flask, and dil. to ca 100 ml with H_2O. Add ca 12 ml H_3PO_4, 5 ml 3% KCN soln, and 15 ml 3% $KMnO_4$ soln, wetting sides of flask with each reagent as it is added. Stopper flasks and mix contents by gentle swirling, wetting entire inside surface. Let stand at least 7 min.; then add ca 2 g solid $FeSO_4.(NH_4)_2SO_4.6H_2O$. Wash down sides of flask and mix. (Clear, nearly colorless soln should result.) If any $KMnO_4$ or MnO_2 remains, add more $Fe(NH_4)_2(SO_4)_2$ (2 g excess does no harm).

Add ca 2 g KI and immediately titr. liberated I with $0.05N$ $Na_2S_2O_3$, using starch indicator. (End point is disappearance of starch-I color; avoid overtitrn as color of soln remains light blue.) 1 ml $0.05N$ $Na_2S_2O_3 = 0.001998$ g Br.

Total Br − inorg. Br = organically combined Br

35.107 Chlorine (*36*)—**Official**

Proceed as in **35.106** until Cl has been driven into halogen absorber. Disconnect absorbing flask, wash contents into 500 ml beaker, and ppt Cl with $AgNO_3$ as in **34.009**. $AgCl \times 0.24737 = Cl$.

Total Cl − inorg. Cl = organically combined Cl.

Chlorine in Presence of Bromine (*36*)—**Official**

35.108 *Method I.*

Proceed as in **35.106** until halogens have been driven into absorbing soln. Disconnect absorption flask, wash contents into 110 ml vol. flask, and dil. to vol. with H_2O. Pipet 50 ml aliquot into 500 ml I flask and det. Br. as in **35.106**. Ppt total halogens in another 50 ml aliquot as in **35.107**.

Calc. wt AgBr equiv. to Br found in first aliquot and subtract from wt ppt obtained from second aliquot. From difference (wt AgCl) calc. Cl present.

$Br \times 3.499 = AgBr$; total $AgCl - AgBr = AgCl$; and $AgCl \times 0.24737 = Cl$.

Multiply results obtained by 2.2 to obtain Cl and Br in the 110 ml soln.

Method II. (*37*)

35.109 REAGENT

Silver iodate.—Suitable for detn of Cl. (Obtainable from Merck & Co., Rahway, N. J.)

35.110 DETERMINATION

Accurately weigh sample contg at least 15 mg Cl or 30 mg Br. Oxidize sample and absorb evolved halogen in mixt. of 15 ml 1% $N_2H_4.H_2SO_4$ *soln* and 5 ml 10% NaOH soln as in **35.106**.

Transfer absorbing soln to 200 ml beaker and wash absorption flask with two 5–10 ml portions H_2O. Complete washing with 5 ml 10% $NaNO_2$ soln and 10 ml H_2SO_4 (1+5), and add these reagents to beaker. Thoroly mix resulting soln and let stand at least 2 min. Wash down sides of beaker with 10 ml *10% sulfamic acid soln* and stir mixt. 2 min. Add excess (0.4–0.8 g) of solid $AgIO_3$, and mix vigorously at least 2 min. Transfer mixt. to 100 ml vol. flask, cool to room temp., and dil. to vol. with H_2O. Mix thoroly and filter thru dry fluted paper. Discard first few ml filtrate.

Dil. aliquot of filtrate to ca 100 ml with H_2O, add 2 g KI, and titr. liberated I with std $Na_2S_2O_3$ soln, **35.103(b)**, using starch soln as indicator. 1 ml $0.1N$ $Na_2S_2O_3 = 0.591$ mg Cl, or 1.332 mg Br.

If sample contains both Br and Cl, dil. soln to exactly 100 ml before adding $AgIO_3$. Neutralize aliquot of this soln, using at least half the soln, with 30% NaOH soln and det. Br as in **35.106**. To remaining soln add solid $AgIO_3$, shake vigorously at least 2 min., filter, and titr. aliquot of filtrate as above to obtain total halide content (mols).

Calc. Br and Cl content of sample from following equations:

$$Br\ (mg) = (T_1 \times N_1 \times 39.96 \times 100)/A;$$

and

$$Cl\ (mg) = [(T_2 \times N_2 \times 100/B) - (3 \times T_1 \times N_1 \times 100/A)] \times 5.91,$$

where T_1 = titrn for Br detn; T_2 = titrn for total halide detn; N_1 = normality of $Na_2S_2O_3$ used in T_1; N_2 = normality of $Na_2S_2O_3$ used in T_2; A = aliquot used for Br detn; and B = aliquot used for total halide detn.

FREE HALOGENS—FIRST ACTION

Free Chlorine or Bromine

35.111 APPARATUS

Two gas-washing bottles (ca 4×25 cm).

35.112 DETERMINATION

Place layer of glass beads in first gas-washing bottle so that top of layer is ca 5 cm above tip of inlet tube, and place 50.0 g sample on top of

beads. Place 5–10 cm layer of glass wool in outlet of bottle to prevent mechanical carry-over of dye.

Place ca 100 ml 1% KI soln contg few drops H_2SO_4 in second gas-washing bottle and connect outlet tube of first bottle to inlet tube of second bottle. Pass steady stream of air thru app. 15–20 min. (CAUTION: Before passing air thru app. be sure that inlet tube of first bottle is not plugged with dye.) Titr. liberated I in second bottle with $0.1N$ $Na_2S_2O_3$, **35.103**(**b**), using starch indicator. 1 ml $0.1N$ $Na_2S_2O_3 = 3.5$ mg Cl or 8.0 mg Br.

INORGANIC SALTS—OFFICIAL

Sodium Chloride in Acid Dyes (*38*)

35.113 REAGENTS

(**a**) *Silver nitrate soln.*—1 ml = ca 0.005 g NaCl. Stdze gravimetrically.

(**b**) *Ammonium thiocyanate soln.*—Stdze by titrn against the $AgNO_3$ soln.

(**c**) *Ferric alum indicator.*—To satd aq. soln $Fe(NH_4)(SO_4)_2$ add just enough HNO_3 to discharge red color.

(**d**) *Activated carbon.*—Norit SG No. 2 or other activated C practically free of Cl or SO_4.

35.114 PREPARATION OF SOLUTION

Add 10 g activated C to soln of 2 g of the dye in ca 100 ml H_2O, and boil gently 2–3 min. Cool to room temp., add 1 ml ca $6N$ HNO_3, and stir vigorously. Transfer mixt. to 200 ml vol. flask, dil. to vol. with H_2O, mix, and filter thru dry paper. If filtrate is colorless, proceed with detn of NaCl and Na_2SO_4. If dye is not completely adsorbed, add 2 g of the C, and stir. Test for complete absorption by dipping corner of piece of filter paper into soln and observing color of liquid that rises in paper. Continue adding C in 2 g portions until test paper no longer shows color (indicating complete adsorption); then filter thru dry paper. Save filtrate for detn of NaCl and Na_2SO_4.

35.115 DETERMINATION

To 50 ml aliquot filtrate in 250 ml g-s. flask add 2 ml ca $6N$ HNO_3, 10 ml of the $AgNO_3$ soln (more if quantity of Cl is large), and ca 5 ml *nitrobenzene*. Shake flask vigorously until AgCl coagulates, add 1 ml of the Fe alum indicator, and titr. excess $AgNO_3$ with the NH_4CNS soln. Take as end point first definite color that persists after shaking 1 min. Calc. NaCl from net $AgNO_3$ titrn on basis of 195 ml total vol., since 10 g C occupies 5 ml.

Net ml $AgNO_3 \times$ NaCl equiv. $\times 195 = \%$NaCl.

Sodium Chloride in Basic Dyes (*38*)

35.116 PREPARATION OF SOLUTION

Dissolve 2 g sample in exactly 200 ml H_2O. Add 10 g activated C, **35.113**(**d**), stir 1 min., and test for complete adsorption as in **35.114**. Add C in 2 g portions, with stirring, until test paper shows no color; then filter thru dry paper.

35.117 DETERMINATION

Evap. 50 ml aliquot filtrate to dryness, and heat to volatilize any NH_4Cl. Transfer residue to 250 ml flask with ca 50 ml H_2O, and det. NaCl as in **35.115**. Net ml $AgNO_3 \times$ NaCl equiv. $\times 200 = \%$ NaCl.

Sodium Sulfate (*38*)

35.118 REAGENTS

(**a**) *Barium chloride soln.*—1 ml = ca 0.0025 g Na_2SO_4. Stdze gravimetrically.

(**b**) *Tetrahydroxyquinone indicator.*—*See* **35.026**(**c**). (In neutral soln contg equal vols alcohol and H_2O, indicator changes from yellow to red when Ba ions are present, and end point is observed by transmitted light. After some practice, results can be reproduced to ± 0.05 ml of the $BaCl_2$ soln. Blank of ca 0.1 ml should be deducted.)

(**c**) *Phenolphthalein indicator.*—0.5% soln in 50% alcohol.

35.119 DETERMINATION

Acid or basic dyes.—Place 25 ml filtrate, **35.114** or **35.116**, in 125 ml erlenmeyer, add 1 drop of the phthln, and make alk. by dropwise addn of ca $0.05N$ NaOH; then add ca $0.002N$ HCl dropwise until indicator is decolorized. Add 25 ml alcohol and ca 0.2 g of the tetrahydroxyquinone indicator. Titr. slowly with the $BaCl_2$ soln, shaking constantly, to red end point. Det. blank on reagents. From net titrn calc. as follows: Acid dyes: Net ml $BaCl_2 \times Na_2SO_4$ equiv. $\times 390 = \%$ Na_2SO_4; Basic dyes: Net ml $BaCl_2 \times Na_2SO_4$ equiv. $\times 400 = \%$ Na_2SO_4.

35.120 Sodium Halides in Halogenated Fluorescein Colors

Place 5 g sample in 400 ml beaker and add ca 150 ml H_2O. (If sample is a color acid, add just enough 10% NaOH soln to give complete soln.) Heat soln nearly to boiling and add 5 ml H_3PO_4. Digest soln until ppt of color acid is well coagulated, cool to room temp., transfer to 250 ml vol. flask, and dil. to vol. Mix thoroly, filter thru dry fluted paper, and discard first few ml filtrate.

(**a**) *Sodium iodide.*—Transfer 100 ml aliquot filtrate to 500 ml tall beaker; add 2.5 ml 30% NaOH soln, few glass beads, and 15 ml satd $KMnO_4$ soln, **35.103**(**a**). Proceed as in **35.104**, beginning "boil 5 min. . . . "

1 ml $0.1N$ $Na_2S_2O_3 = 0.002498$ g NaI.

(**b**) *Sodium bromide.*—Place 100 ml aliquot filtrate in 500 ml I flask and proceed as in **35.106**, beginning "Add ca 12 ml H_3PO_4 . . . "

1 ml 0.05N $Na_2S_2O_3$ = 0.00257 g NaBr.

(c) *Sodium chloride.*—Place 100 ml aliquot filtrate in 400 ml beaker, heat to boiling, and add enough 10% $AgNO_3$ soln to ppt halides. Digest soln until ppt is well coagulated, cool, and transfer ppt to weighed gooch. Wash ppt thoroly with H_2O and alcohol. Dry crucible and contents at 135°, cool, and weigh.

If sample contains no NaBr or NaI, NaCl content can be calcd directly from wt ppt. If other halides are present, deduct wt Ag halide equiv. to I or Br found, (a) or (b), from wt ppt before calcg NaCl content.

$AgCl \times 0.4078 = NaCl$; $NaI \times 1.5662 = AgI$; and $NaBr \times 1.8248 = AgBr$.

Sodium Acetate

35.121 APPARATUS

App., Fig. 71, can be assembled from stock items. Distg flask is 125 ml acetylation flask with ₸ 24/40 joint. Receiver is 300 ml flask.

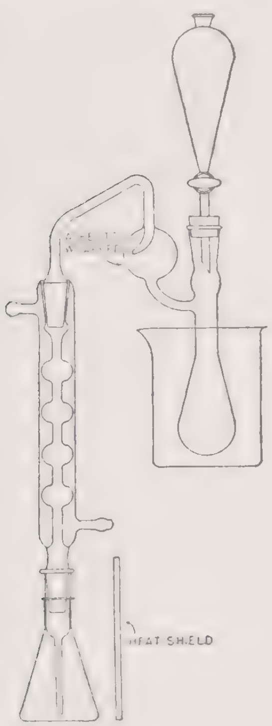

FIG. 71.—SODIUM ACETATE APPARATUS

35.122 REAGENTS

(a) *p-Toluenesulfonic acid.*—Dry p-toluenesulfonic acid monohydrate overnight at 110°, cool, and grind to powder.

(b) *Silver toluenesulfonate.*—Dissolve Ag_2O or Ag_2CO_3 in ca 10% excess of p-toluenesulfonic acid soln, evap. to dryness, and dry 8 hr at 135°.

(c) *m-Cresol purple indicator.*—Triturate 0.5 g m-cresolsulfonphthalein with 13 ml 0.1N NaOH and dil. with H_2O to 100 ml.

35.123 DETERMINATION

To 500 ml erlenmeyer add 100 ml H_2O, 1 drop of the m-cresol purple indicator, and enough 0.1N NaOH or 0.1N HCl to turn color of soln just yellow; place flask under condenser.

Transfer 30 ml absolute alcohol to distn flask and add thru powder funnel 5.00 g sample, 5 g of the p-toluenesulfonic acid, and 1 g of the Ag toluenesulfonate. Add 3–4 pieces Alundum or other anti-bumping agent and mark level of liquid in distn flask. Wash funnel and neck of flask with 25 ml absolute alcohol. Shake flask to mix contents thoroly and attach it to condenser.

Immerse distn flask as far as possible in beaker of hot H_2O and heat H_2O to boiling. After ca 25 ml distillate collects, remove heat source and slowly add 25 ml absolute alcohol to distn flask. When contents of flask again begin to distill gently, replace heat source and collect second 25 ml distillate. Make third addn of alcohol and distill in similar manner. Finally boil until distn rate is slow (ca 30 min. for total time from beginning of first distn).

Wash 50 ml H_2O down condenser into receiver, and add 50.0 ml 0.1N NaOH. Add 3–4 pieces Alundum or other anti-bumping agent and connect to reflux condenser fitted with absorption tube contg Ascarite or other CO_2-absorbing material. Reflux 10 min., cool to room temp., add few drops of the m-cresol purple indicator, and titr. with 0.1N HCl to yellow-green that does not change in hue on further addn of acid. Det. blank by repeating procedure, omitting sample.

Calc. NaOAc from net vol. std NaOH soln required. 1 ml 0.1N NaOH = 0.0082 g $C_2H_3O_2Na$.

SOLUBLE MATTER—FIRST ACTION

35.124 Water-Soluble Matter

Place 5 g well-powd. sample in 500 ml erlenmeyer or wide-mouth bottle, add 200 ml H_2O, stopper, and shake vigorously. Repeat mixing several times during 2 hr period. Filter mixt. and evap. 100 ml filtrate in weighed Pt dish on steam bath. Dry in oven at 100–105°, cool in desiccator, and weigh. Report increase in wt as H_2O-sol. matter. Test small portions of remainder of filtrate for chlorides, sulfates, and nitrates. If more than traces are present, make proper analyses on aliquot portions of filtrate.

35.125 Chloroform-Soluble Matter

Weigh 5 g sample into cellulose thimble and ext. in Soxhlet app. with $CHCl_3$ 16 hr. Transfer ext. to separator and wash with 30 ml portions H_2O until washings are practically colorless. Ext. combined washings with ca 30 ml $CHCl_3$ and add washings to main ext. Drain $CHCl_3$ ext. into

weighed dish. Wash separator with few ml $CHCl_3$ and add washings to dish. Evap. at room temp. and dry in desiccator to constant wt (±0.5 mg), weigh, and report as $CHCl_3$ ext.

35.126 Matter Soluble in One Per Cent Aqueous Hydrochloric Acid

To 2 g sample in 300 ml beaker add 100 ml 1% HCl (1+99). Boil gently 5 min., stirring continuously, cool, and filter thru dry paper. Place 50 ml filtrate in previously weighed dish, evap. nearly to dryness, dry in oven 2 hr at 105°, cool in desiccator, and weigh. Increase in wt is matter sol. in 1% HCl.

Melting Point—Official

35.127 APPARATUS—*See* Fig. 72

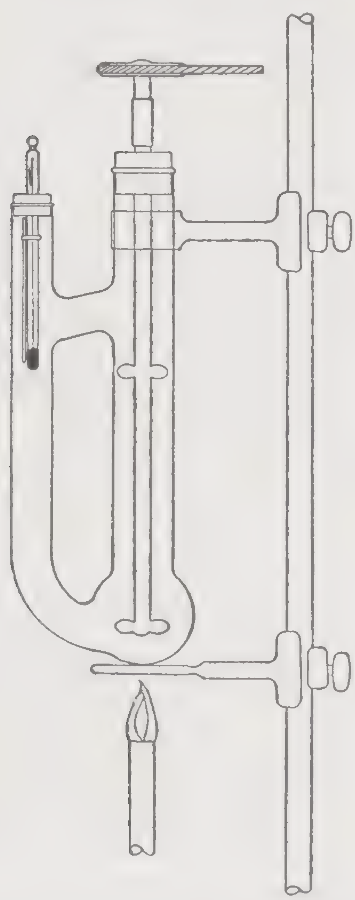

FIG. 72.—MELTING POINT APPARATUS

35.128 DETERMINATION

To capillary tube 1 mm or smaller i.d., sealed at one end, transfer small portion of sample by inserting open end of tube into sample, removing tube, inverting, and gently tapping until loosely packed substance fills bottom of tube to height of 2–4 mm. Attach tube to thermometer so that sample is placed at ca middle of Hg bulb. Raise temp. of bath rapidly to within 5° of approx. m. p. of sample; then raise slowly until melting is observed. When temp. rises to ca 0.5° of m. p., substance usually darkens; true melting is indicated by formation of meniscus on upper surface. When this condition is observed, record temp., and consider this temp. as m.p. of sample. Keep temp. as nearly constant as possible until entire sample liquefies; then record temp. again.

35.129 Free Acid—First Action

Dissolve 1 g sample in exactly 10 ml H_2O, and det. pH of soln. If pH is 4.7 or more, no free H_2SO_4 is present.

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(*2*) U. S. Dept. Agr. Bur. Chem. Bull. **448**; U. S. Dept. Agr. Bur. Chem. Circs. **25** and **63**; Allen, "Commercial Organic Analysis," 4th ed., 1911, Vol. 5; Leach-Winton, "Food Inspection and Analysis," 4th ed., 1920; Girard, "Analyse des Matières Alimentaires et Recherche de leurs Falsifications," 2nd ed., 1904; U. S. Dept. Agr. Bur. Animal Ind. Circ. **180**; J. Ind. Eng. Chem. **8,** 1123(1916); **9,** 955(1917).

(*3*) U. S. Dept. Agr. Bur. Chem. Bull. **65,** p. 152; Ann. fals. **3,** 293(1910); U. S. Dept. Agr. Bur. Chem. Circs. **25** and **63**; Abs. Chem. Centr. **69,** (*2*) 943(1898); J. Ind. Eng. Chem. **8,** 614(1916); **10,** 436(1918).

(*4*) Abs. Z. anal. Chem. **26,** 100(1887); **27,** 232 (1888).

(*5*) Federal Security Agency, S.R.A., F.D.C. 3 (1940); Compilation of Regulations for Color Certification, U S. Dept. Health, Education, and Welfare (1959).

(*6*) U. S. Dept. Agr. Bull. **1390,** Supplement 1 (1930).

(*7*) J. Assoc. Offic. Agr. Chemists **34,** 453 (1951).

(*8*) Ibid. **35,** 423(1952); **36,** 802(1953).

(*9*) U. S. Dept. Agr. Bur. Chem. Circs. **25** and **63**; Allen, "Commercial Organic Analysis," 4th ed., **5,** 625 (1911); Leach-Winton, "Food Inspection and Analysis," 4th ed., 1920.

(*10*) Rupe, "Die Chemie der Natürlicheu Farbstoffe," 1900.

(*11*) Perkin and Everest, "The Natural Organic Coloring Matters," 1918.

(*12*) Willstatter and Stoll, "Untersuchungen über Chlorophyll, Methoden und Ergebnisse," 1913.

(*13*) Sitz. preuss. Akad. Wiss. **12,** 402(1914); Ann. **401,** 189(1913); **408,** 1(1915); **412,** 164, 195 (1916); Ber. pharm. Ges. **25,** 447(1915); Ber. **51,** 782, 784(1918).

(*14*) Molisch, Ber. botan. Ges. **14,** 16(1896).

(*15*) Leach-Winton, "Food Inspection and Analysis," 4th ed., 1920, pp. 161, 558.

(*16*) Girard, "Analyse des Matières Alimentaires et Recherche de leurs Falsifications," 2nd ed., 1904.

(*17*) Mass. Inst. Tech. Quart. **21,** 280(1908).

(*18*) J. Assoc. Offic. Agr. Chemists **24,** 904 (1941); **32,** 644(1949).

(*19*) Ibid. **26**, 182(1943).
(*20*) Ibid. **27**, 576(1944); **30**, 522(1947); **31**, 598, 674(1948); **32**, 130, 635(1949).
(*21*) Ibid. **25**, 956(1942); **26**, 242(1943); **27**, 113(1944).
(*22*) Ibid. **25**, 936(1942).
(*23*) Ibid. **31**, 592(1948); **32**, 613(1949).
(*24*) Ibid. **31**, 594(1948); **32**, 624(1949).
(*25*) Ibid. **35**, 419(1952).
(*26*) Ibid. **33**, 398(1950); **34**, 407(1951).
(*27*) Ibid. **32**, 640(1949).
(*28*) Ibid. **33**, 937(1950).
(*29*) Ibid. **32**, 672(1949).
(*30*) Ibid. **37**, 805(1954).
(*31*) Ibid. **35**, 754(1952).
(*32*) Ibid. **30**, 552(1947); **32**, 622(1949).
(*33*) Ibid. **31**, 677(1948); **32**, 621(1949).
(*34*) Ibid. **25**, 755(1942); **32**, 680(1949).
(*35*) Ibid. **26**, 433(1943); **28**, 757(1945).
(*36*) Ibid. **26**, 433(1943); **32**, 609(1949).
(*37*) Ibid. **32**, 680(1949).
(*38*) Ibid. **25**, 958(1942).

36. Extraneous Materials: Isolation

36.001 APPARATUS

(a) *Blood counting cell.*—Depth 0.1 mm, preferably ruled in the Thoma or old Neubauer system. Cell with "improved" Neubauer system which is equipped with optically worked cover glass may also be used.

(b) *Bolting cloth.*—Silk cloth woven to std size opening and thickness which is used in flour mills. Number of the silk specifies number of mesh per linear inch. "X," "XX," or "XXX" after number refers to thickness of thread from which cloth is woven and this also affects size of opening in cloth. Therefore, follow designation exactly as to both number and "X" of bolting cloth.

Prep. disks by boiling large squares of silk before cutting them into circles. Circles cut from unboiled silk shrink and become misshapen. Make rulings ca 5–7 mm apart with India ink or other permanent marking material, using fine pen, on boiled and pressed cloth marked off in circles ca 85 mm diam.

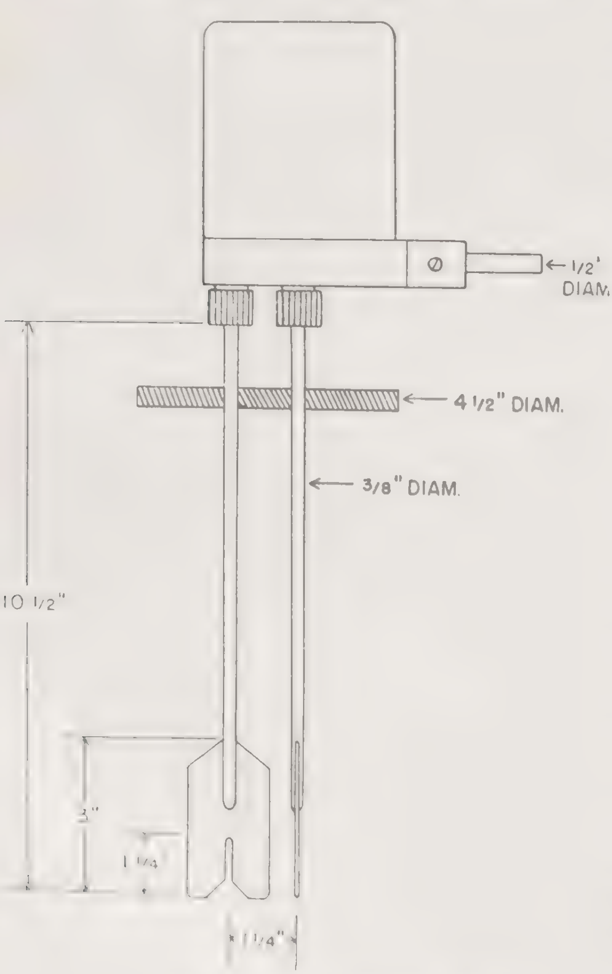

FIG. 73.—MECHANICAL BUTTER STIRRER

(c) *Butter stirrer.*—See Fig. 73.

(d) *Compound microscope.*—*See* (k)(1).

(e) *Cyclone.*—Laboratory cyclone or pulper consists of cylindrical perforated metal screen in which revolves paddle which forces soft material from food product out thru openings in screen. Tough materials such as seeds, skins, and stems are moved along and out opening in end of cylinder. Use as power source $\frac{1}{4}$ horsepower, 110 volt, 1725 rpm elec. motor. Screen is 22 gauge material, 400 holes per sq. in., each 0.027″ diam. Screen is 2.5″ i.d. and length of effective screen is 3″. Paddle has 2 fins, each 25/32″ wide, set alternately and extending $1\frac{3}{16}$″ from center of shaft. Pulper is fed thru hopper which leads into basin 3.5″ long and 2.5″ i.d. Portion of paddle with fins inserted at 30° angle forces material from basin into screening compartment. Cyclone is so constructed that waste opening may be closed, as needed. Sieved material is caught in shield and delivered thru spout to container. Machine may be readily disassembled for washing. (Available from Cefaly Experimental Co., 3502 Perry St., Brentwood, Md.)

(f) *Filter paper.*—Use smooth, high wet-strength, rapid-acting filter paper ruled with oil-, alcohol-, and water-proof lines 5 mm apart. Satisfactory paper which meets requirements except high wet-strength is S&S No. 8.

(g) *Funnels for filtration with suction.*—Büchner or Hirsch funnels can be used interchangeably. Use funnels with filter papers or bolting cloth cupped up on sides to eliminate loss of solids. (For filtration thru Hirsch funnel or büchner, use of suction and rapid-acting filter paper is assumed.)

Use of wire screen or bolting cloth between perforated funnel plate and filter paper accelerates filtration and causes more uniform distribution of solids.

(h) *Greenough-type (widefield stereoscopic) microscope for filth examination.*—*See* (k)(2).

(i) *Illuminators for microscopic work.* Three types of illuminators are used: 2 for widefield stereoscopic and 1 for compound microscope.

(1) *Illuminator for widefield stereoscopic microscope for filth examination.*—Illuminator for this purpose should have: Compactness and flexibility; transformer or resistor to vary light intensity;

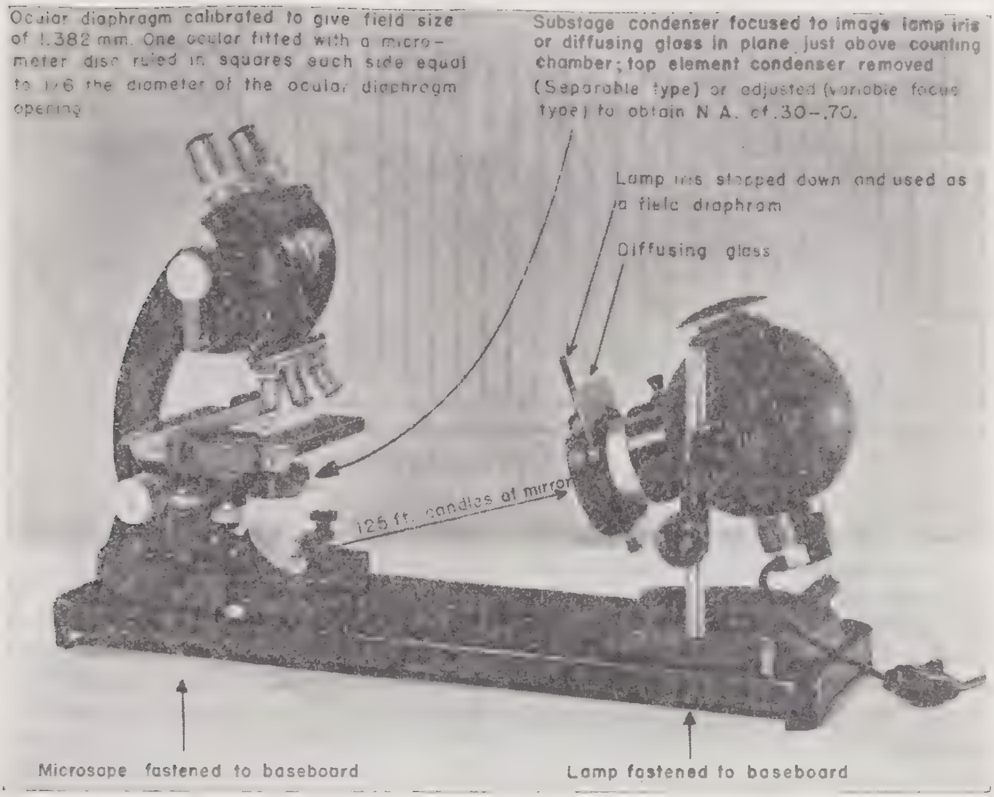

FIG. 74.—COMPOUND MICROSCOPE FOR MOLD COUNTING

focusing adjustment to give uniformly lighted field of view; blue-white color from cool low-voltage source. One illuminator which fulfills these requirements is Bausch and Lomb, "Reflector Illuminator," No. 31-33-11-01, when equipped with clear blue bulb. If bulbs are not clear, light cannot be focused.

(2) *Illuminator for widefield stereoscopic microscope for rot fragment counting.*—Use small substage illuminator fitted with daylight or blue ground-glass filter and 15 watt bulb. Bausch and Lomb No. 31-33-13-01 or 31-33-13-03, or American Optical Co. No. 385A or 385B with 15 watt bulb is satisfactory.

(3) *Illuminator for compound microscope.*—Satisfactory illuminator for compound microscope should have: Compact and sturdy construction; iris diaphragm external to condensing lenses; condensing system for focusing light; well-ventilated lamp housing; arrangement for holding filters; adjustable stand for both height and inclination. American Optical Co. No. 370A or Bausch and Lomb No. 31-33-75-51 fits above requirements.

(j) *Howard mold-counting apparatus.*—(1) *Howard mold-counting slide.*—Glass slide of one-piece construction with flat plane circle ca 19 mm diam. or rectangle 20×15 mm surrounded by moat and flanked on each side by shoulders 0.1 mm higher than plane surface. Cover glass is supported on shoulders and leaves depth of 0.1 mm between underside of cover glass and plane surface. Central plane, shoulders, and cover glass have optically worked surfaces. To facilitate calibration of microscope, newer slides are engraved with circle 1.382 mm diam. or with 2 fine parallel lines 1.382 mm apart.

(2) *Accessory disk for mold-counting.*—Glass disk that fits into microscope eyepiece, ruled into squares each side of which is equal to $\frac{1}{6}$ of diam. of field. Since limiting diaphragm is eyepiece field stop, rulings equal $\frac{1}{6}$ of this diaphragm opening. Field viewed on slide with mold-counting microscope has diam. of 1.382 mm at magnification of 90–125×.

(3) *Method of illumination of compound microscope for mold counting.*—*See* **36.003**(h)(11). Fasten lamp and microscope securely to baseboard so that they are used and maintained as unit (*e.g.*, Bausch and Lomb baseboard No. 31-50-54, Fig. 74). Adjust mirror pivot so that it is not easily moved, and hold microscope in place by screws or cleats.

(k) *Microscopes.*—(1) *Compound microscope.*—For mold counting and other filth and decomposition work, microscope should have following min. specifications: Binocular body with inclined oculars; 4 parfocal achromatic objectives of ca 5, 10, 20, and 40×; revolving 4-place nosepiece; achromatic condenser with N.A. of 1.40 in

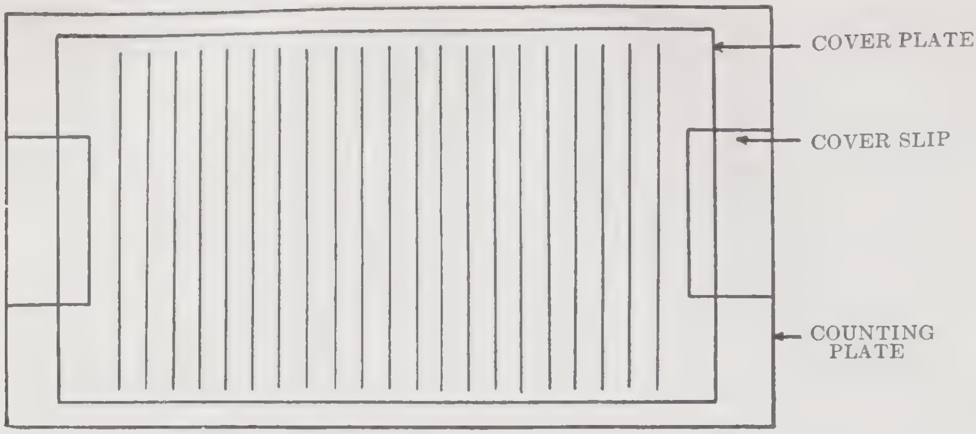

FIG. 75.—ROT FRAGMENT COUNTING SLIDE

centerable mount; 10× Huygenian eyepieces; fine adjustment; mechanical stage preferably with adjusting buttons at sides of stage (if photomicrographs are to be made, revolving stage is more satisfactory); stdzd field of view of 1.382 mm diam. at 90–125×; equipped with drop-in ocular disk ruled in squares, each of which is $\frac{1}{5}$ of field diam. *See* Fig. 74.

(2) *Widefield stereoscopic microscope for filth examination.*—Microscope for filth work should have following min. specifications: Binocular body with inclined oculars; sliding or revolving nosepiece to accommodate 3 objectives; 3 parfocal objectives of 1×, 3×, and 6 or 7.5×; paired 10× and paired 15× widefield oculars; prism housing geared together so that rotation of one eyepiece moves other; mounted on base and capable of illumination by transmitted or reflected light. Two suitable widefield microscopes are Bausch and Lomb BKT and American Optical Co. 25LG.

(l) *Petri dishes.*—Use to hold filter papers, bolting cloths, etc., for microscopic examination; low-edge (10 mm high) type.

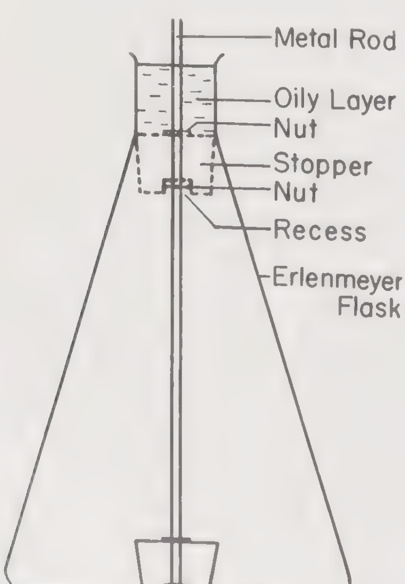

FIG. 76.—WILDMAN TRAP FLASK

(m) *Rot fragment counting plate and cover.*—Glass plate 1.5–4.0 mm thick with covers 50×85 mm, ca 1.5 mm thick. Dimensions of plate: 55×100 mm; rulings: crosswise, parallel lines 4.5 mm apart, with 15 mm space at each end. Fasten $\frac{1}{2}$ of square coverslip, ca 22 mm on side and ca 0.25 mm thick, at each end of counting plate to raise cover plate above ruled plate. *See* Fig. 75.

(n) *Sieves.*—*See* Definitions of Terms and Explanatory Notes.

(o) *Trap flask (Wildman).*—Consists of 1 or 2 L erlenmeyer into which is inserted close-fitting rubber stopper supported on stiff metal rod $\frac{3}{16}''$ diam. and ca 4″ longer than height of flask. (Rod of greater diam. is not desirable because of its greater displacement of liquid.) Rod is threaded (#10–32) at lower end and furnished with nuts and washers to hold it in place on stopper. Countersink lower nut and washer in the rubber to prevent striking flask. *See* Fig. 76 and **36.003(a)**.

(p) *Water filters.*—Where ample continuous supply of filtered H_2O is needed, install permanent arrangement. Two filters that may be constructed from materials available in most laboratories are described below and illustrated in Fig. 77.

(1) Constructed from flared test tube held to bottom non-splash portion of aspirator by screw cap or metal ring that normally holds wire screen on aspirator. Filter disk is supported on wire screen and when flange of test tube is tightened against disk, sufficiently tight seal is obtained.

(2) Constructed from garden hose female coupling with wire screen and 2 filter disks placed between 2 thin washers so that when used on threaded faucet spout assembly is sealed. Washers may be cut from rubber or plastic, and disks may be cut from Credicott sediment cloth, or filter paper disks may be used. For wire disks, No. 100 sieve is preferred.

With either device (1) or (2), disks may be re-

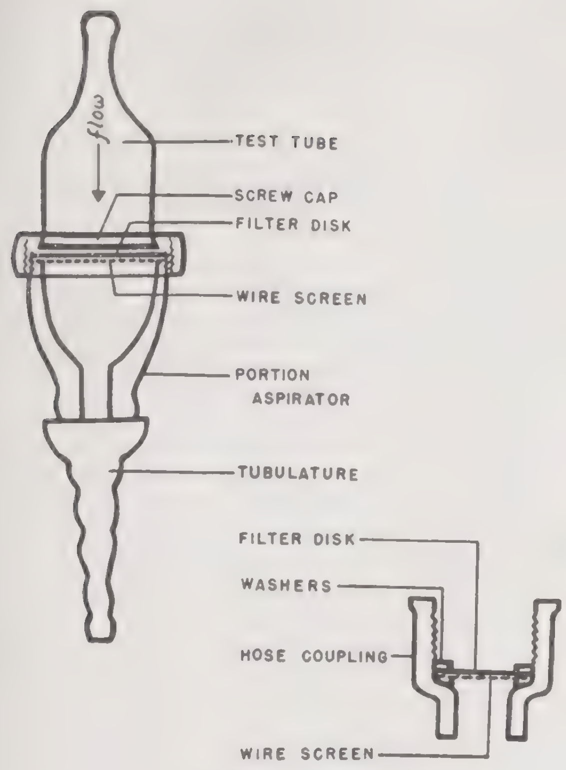

FIG. 77.—FILTERS

moved at regular intervals for examination. Glass tube affords ready means of observing quantity of sediment collecting on filter.

36.002 REAGENTS

(**a**) *Acetic acid.*—Practical glacial HOAc is satisfactory.

(**b**) *Acetone.*—Practical acetone is suitable unless otherwise specified.

(**c**) *Alcohol.*—95% commercial ethanol (not denatured) unless otherwise specified. Make all dilns by vol.

(**d**) *Algin soln for rot fragment determination.*—Proceed as for stabilizer solns (**aa**); then adjust final mixt. to pH 7.0–7.5 with NaOH soln.

(**e**) *Borax.*—Household borax is satisfactory. This is usually $Na_2B_4O_7.10H_2O$ which is not very sol. in cold H_2O. If >5% borax soln is desired, it must be kept hot.

(**f**) *Calgon.*—Na hexametaphosphate (obtainable from Calgon, Inc., Pittsburgh, Pa. adjusted with added alkali).

(**g**) *Carbon tetrachloride.*—Tech. grade is suitable unless otherwise specified.

(**h**) *Carob bean soln.—See* (**aa**).

(**i**) *Castor oil.*—USP.

(**j**) *Chloral hydrate.*—Aq. soln (1+1) or *see* Hertwig's soln.

(**k**) *Chloroform.*—Tech. grade is suitable unless otherwise specified. Mixt. of petr. ether and CCl_4 adjusted to sp. gr. of $CHCl_3$, 1.5, may be used instead of $CHCl_3$.

(**l**) *Chloroplatinic acid soln.*—Dissolve 5 g H_2PtCl_6 in 100 ml H_2O.

(**m**) *Crystal violet soln.*—Dissolve 5 g dye (Colour Index 42555) in 100 ml HCl (1+2.5) and filter. Test soln as in **36.063**; if blue color does not develop, add NaOAc, small amount at time, until color develops under conditions of **36.063**.

(**n**) *Formaldehyde soln.*—Use NF soln. Strength is expressed as % by vol. of NF soln.

(**o**) *Gasoline.*—Use any Pb-free gasoline except those contg benzene. Amoco is not suitable.

(**p**) *Hertwig's soln.*—Useful for clearing plant and insect materials; action continues on standing. Use only for temporary mounts; not for permanent slides. Mix 19 ml HCl, 150 ml H_2O, 60 ml glycerine, and 270 g chloral hydrate.

(**q**) *Isopropyl alcohol saturated with gasoline.*—Dissolve 150 ml gasoline per L tech. isopropyl alcohol. Mix and dil. the alcohol to 60% with H_2O. Let stand until almost clear and withdraw by siphoning from beneath gasoline layer.

(**r**) *Kerosene.*—Ordinary commercial grade.

(**s**) *Mineral oil.*—Use light mineral oil, USP.

(**t**) *Pancreatin soln.*—Use NF or sol. pancreatin kept refrigerated at ca 10°. Use fresh soln. Mix 10 g pancreatin with 100 ml warm H_2O (not >40°). Stir with malted milk mixer 10 min., or let stand 30 min. with intermittent stirring. Pour soln thru 4″ loosely packed pad of cotton in 4–5″, 60° funnel. Repeat filtration thru same pad. If filtering is slow in either filtration, change cotton. Filter with suction thru fast paper in büchner or Hirsch funnel. If filtration is slow, pour soln thru slightly compressed cotton plug in the 60° funnel. Repeat if necessary until soln filters rapidly thru paper. (Sol. pancreatin may be filtered directly thru paper with suction.) Dil. filtrate to 100 ml for each 10 g portion.

(**u**) *Phosphoric acid.*—Tech. grade.

(**v**) *Sodium carbonate.*—Tech. grade. If hydrated salt is used, calc. to anhyd. basis.

(**w**) *Sodium chloride.*—Tech. grade.

(**x**) *Sodium oleate.*—Tech. grade.

(**y**) *Sodium phosphate soln.*—Tech. grade Na_3PO_4. Prep. 5% soln.

(**z**) *Sodium sulfite.*—Tech. grade. If hydrated salt is used, calc. to anhyd. basis.

(**aa**) *Stabilizer solns.*—3–5% Pectin or 1% algin. Add required quantity of stabilizer directly to H_2O while agitating in high speed blender. Treat soln with vac. or heat to remove air bubbles. Add 2 ml HCHO/100 ml soln as preservative. (If blender is not available, mix dry stabilizer with alcohol to facilitate incorporation with H_2O.) Adjust to pH 7.0–7.5.

(**bb**) *Tween 80–60% alcohol soln.*—To 40 ml polyoxyethylene sorbitan monooleate (Atlas Powder Co., Wilmington, Del.) add 210 ml 60% alcohol, mix, and filter. (Proportionate reagent quantities may be prepd.)

(cc) *Urease.*—25 mg tablets.

(dd) *Versene soln.*—Tetrasodium salt of ethylenediaminetetraacetic acid (Na_4EDTA). Dissolve 5 g salt in 100 ml H_2O, add 150 ml alcohol, mix, and filter. Proportionate reagent quantities may be prepd.

36.003 SPECIAL TECHNICS

(a) *Operation of Wildman trap flask.*—Unless otherwise directed in specific method, cool mixt. in flask to room temp. Bring vol. of liquid to ca 900 ml in 2 L flask and to ca 600 ml in 1 L flask. Add gasoline or oil as stated in method. Tilt flask ca 45° from vertical and mix 1 min. at rate of 200–250 strokes/min. with brisk rotary motion so that liquid is brought to a roll. Avoid splashing thru surface of liquid with rubber stopper. Add enough liquid to bring oil layer well into neck of flask.

Unless otherwise stated, let mixt. stand 30 min., intermittently stirring bottom layer every 3–6 min. during first 20 min. of standing. Spin stopper to remove sediment and trap off by raising stopper as far as possible into neck of flask, being sure that oil layer and at least 1 cm of liquid below interface are above stopper. Hold stopper in place and pour off liquids into beaker. Rinse out material on rod and in neck of flask with extn liquid in which flotation was performed and add to beaker.

Do not use 95% alcohol or other liquid to wash out neck of flask which may interfere with surface relationship of the 2 phases; this will cause loss in recovery in subsequent trappings. Filter trapped material and rinsings with suction thru rapid paper in büchner or Hirsch funnel. Add gasoline or oil, as specified, to trap flask and stir vigorously. Add enough flotation liquid to bring oily layer into neck of flask. Trap off again, rinse, and filter as above.

(b) *Filtration technic.*—(Treatment of trapped-off material.) If material trapped off in beaker contains appreciable starchy debris, add enough HCl to make soln 1–2% in HCl (1+99–49), bring to boil, and filter while hot. If fats or colloidal material retard filtration, hasten by playing stream of hot H_2O over paper during filtration.

(c) *Clearing of plant materials.*—With sedimentation or flotation procedures some food material may be trapped off with filth particles. By proper clearing, filth may be made to stand out in contrast with white background of filter paper by use of one of following procedures:

(1) For heavy filth, moisten paper with H_2O or 50% alcohol. (This method does not clear material completely, but it leaves rodent pellets and other filth soft and pliable.)

(2) For light filth examination, wet paper with glycerine-alcohol (1+1) immediately after filtering. Place enough liquid on paper to fill fibers but not enough to cause flowing of extd materials. This clearing agent does not harden filth material on paper, as do many oils which might be used as clearing agents.

(3) Clove oil can be used for clearing plant materials. This oil has high refractive index and clears more completely than does alcohol-glycerine soln.

(d) *Clearing for examination under compound microscope.*—Chloral hydrate solns completely clear and gelatinize most plant materials. Use for quick clearing of temporary mounts. Since action continues during storage, do not use for permanent mounts.

(e) *Preparation of bolting cloth disks.*—*See* **36.001(b)**.

(f) *Illumination for the widefield stereoscopic microscope.*—(1) *For examining filter papers.*—Focus and adjust light to strike paper at ca 70° angle from horizontal. Light may come from right or left.

(2) *By transmitted light.*—In cases where transmitted light is necessary, use mirror on microscope stand. Mirror with white surface instead of conventional silvered mirror is particularly useful.

In counting rot fragments, remove mirror and metal contrast plate and replace with box-type substage lamp. Place lamp, **36.001(i)(2)**, so that center of glass filter is directly below objective and within 2 cm of glass microscope plate.

(g) *Microscopic examination of filter papers.*—Make examination at 30× (unless otherwise specified), using widefield stereoscopic microscope, on properly cleared paper. Continual teasing and probing of the food particles while observing thru microscope is essential. Turn over all large pieces of material such as bran which might obscure filth elements. Examine all doubtful pieces of material at 60–75×. At least twice magnification used in original examination is necessary to show new details not observable at lower power. If doubt still remains, mount piece, clear thoroly, and examine under compound microscope. *Thoro knowledge of appearance of authentic materials is assumed.*

(h) *Illumination of compound microscope.*—(1) Mount lamp permanently with ground glass or filters at least 10″ from mirror.

(2) Center bulb filaments with lamp lens. (If bulb and housing are permanently aligned, this step is unnecessary.) Remove filters or ground glass. Close lamp iris and observe filaments on wall perpendicular to lamp axis. If either less or more than number of filaments in bulb are seen, bulb is not centered and must be adjusted by twisting base socket or moving it up or down. Image of 1 coil, when multiple coil bulb is used, indicates that filament should be placed more nearly perpendicular to lamp axis. Image of more

coils than are present in bulb indicates that bulb is too high or too low. It is usually impossible to completely eliminate all multiple filaments but final adjustment should show the number of spiral filaments in bulb prominently and with min. amount of light in secondary filaments.

(3) Center image of bulb filament on microscope mirror, partly closing lamp diaphragm to obtain sharper image of filaments.

(4) Focus image of bulb filament on microscope diaphragm either by moving position of bulb by means of set screw on side of lamp, or by adjusting lamp condensing system.

(5) Direct light thru microscope by tilting mirror. Check roughly by looking thru eyepiece; then open lamp diaphragm wide and add filters or reduce light intensity with voltage control to avoid intense glare. Insert ground glass in rack provided at front of lamp.

(6) Focus microscope on specimen. To prevent damage to objective and slide, make all downward adjustments while observing slide and objective. Bring specimen into focus by moving objective away from slide.

(7) Focus microscope condenser. (With variable focus condenser, steps which follow should be ignored and manufacturer's directions for use of this type of condenser should be followed.) Close lamp diaphragm to smallest opening and focus microscope condenser by racking it up or down until lamp diaphragm, seen thru microscope, is at its smallest and sharpest. It may be necessary to tilt mirror slightly to place image of diaphragm in center of field. After it is focused, rack condenser slightly higher until ground glass background is no longer visible. If it is not possible to rack condenser higher, lower it until same effect is achieved.

(8) Center condenser. Open lamp diaphragm to wide aperture and close condenser diaphragm to smallest opening. *For this adjustment, top element of the condenser must be on.* Rack microscope up until image of condenser diaphragm can be seen. With knurled adjusting screws, adjust condenser until image of closed diaphragm is in center of field. This is not most accurate method of centering condenser but serves for most purposes. Further check on centering may be made by re-focusing microscope on specimen. Open condenser diaphragm slightly, close lamp diaphragm (if necessary, readjust mirror to bring light spot into center of field), and observe color fringe around outline of image of lamp diaphragm. If light has uniform color fringe, condenser is centered. If color fringe is not uniform, again check condenser for centering.

(9) Adjust size of lamp diaphragm to coincide with field of view. This step aids in reducing glare from stray light.

(10) Close condenser diaphragm to give added contrast, which is necessary in mold counting and for making photomicrographs of clear pieces of insect fragments. Since closing condenser decreases resolution and increases diffraction, however, compromise is necessary.

(11) For mold counting, with microscope adjusted as above, remove top element of condenser and re-focus condenser as in step (7). This is the only change necessary for mold counting.

BEVERAGES AND BEVERAGE MATERIALS

Canned Citrus and Pineapple Juices

36.004 Mold Count—First Action

Pour contents of can into beaker and mix thoroly by pouring back and forth between beaker and can at least 12 times. After mixing, transfer 50 ml juice to graduated 50 ml conical-bottom centrifuge tube. Centrifuge 10 min. at 2200 rpm, using International type SB, size 1 centrifuge, with 8-place No. 240 head (distance from center of centrifuge head to center of cups (at rest) is $5\frac{1}{4}''$), or other centrifuge giving equiv. centrifugal force as computed by following formula: $N_1^2r_1 = N_2^2r_2$, where N = rpm, and r = radius of centrifuge arm. Check speed with tachometer, since rheostat does not necessarily indicate speed in rpm.

Let centrifuge gradually come to complete stop before removing tubes and read vol. sediment in centrifuge tube. Remove tube and decant supernatant without disturbing sediment. With pineapple juice, add 0.5 ml HCl (to dissolve oxalate crystals). Add H_2O to tube to bring level to 10 ml mark and then add 5 ml 3% pectin soln, **36.002(aa)**. Thoroly mix sediment, H_2O, and pectin soln and pour into small beaker. Mix by pouring back and forth between beaker and tube at least 6 times. Stir mixt. thoroly in beaker and proceed as in **36.060.** In addition to checking microscopic fields, indicate those fields positive due to Oöspora.

36.005 Fly Eggs and Maggots—First Action

Filter 250 ml thoroly mixed sample thru büchner fitted with 10XX bolting cloth (wire mesh screen under bolting cloth facilitates filtration). Pour juice slowly to avoid accumulation of excess pulp on cloth (2 or 3 cloths may be necessary). Examine filters microscopically.

36.006 Insect Fragments and Rodent Contamination—First Action

To 250 ml juice in 2 L trap flask add 15 ml castor oil, and fill with enough hot H_2O (ca 70°), stirring vigorously, to bring oil layer into neck of

flask. Let stand 30 min. Trap off, filter, and examine.

Cocoa, Chocolate, Chocolate Liquor, Imitation and Substitute Chocolate Products

36.007 Filth—First Action

Examine by **36.056(c)**.

36.008 *Ground Coffee and Coffee Substitutes—First Action*

Weigh 100 g in 600 ml beaker, add 350 ml $CHCl_3$, and boil 15 min., stirring occasionally. Wash down sides of beaker with $CHCl_3$. Let mixt. cool and settle 15 min. with occasional stirring of top layer. Decant $CHCl_3$ and floating tissue onto smooth ca 15 cm filter paper in büchner, taking care not to disturb heavy residue on bottom of beaker. Repeat decanting with small quantities of $CHCl_3$ until practically no plant tissue remains with residue on bottom of beaker. (Sp. gr. of $CHCl_3$ may be increased by addn of CCl_4, if necessary to float plant tissue. Do not add CCl_4 beyond 1 part CCl_4 to 1 part $CHCl_3$.) Transfer residue from beaker to ashless filter paper and examine for filth. If residue is appreciable, ignite filter and det. wt sand, soil, etc.

Air dry decanted material on paper overnight or for 1 hr in oven at ca 80°, transfer dried material to 2 L trap flask, and add 400 ml hot H_2O. Boil 15 min. and, if necessary, add small amounts cold H_2O intermittently to prevent foaming. Cool mixt. to <20°. Trap off twice, using 35 ml and 25 ml portions gasoline, resp. In first trapping, after stirring gasoline let stand 5 min. before filling flask. Filter and examine microscopically.

DAIRY PRODUCTS

Sediment Test on Milk—Official

36.009 APPARATUS AND MATERIALS

(**a**) *Tester.*—Simply constructed, easily cleaned, and adjustable between samplings to permit sanitary removal of used disk and replacement with clean disk. Before using, check tester for reproducibility as in **36.010.** Milk or sediment must not by-pass disk. Select type according to method of sampling:

(1) *For mixed sample method.*—Pressure, gravity, or vac. type: (a) For 1 gallon sample use any suitable device that will filter sample thru disk with exposed area $1\frac{1}{8}''$ diam. (b) For 1 pint sample, equip single-unit, off-bottom tester with special head (available from Sediment Testing Supply Co., 20 E. Jackson Blvd., Chicago, Ill.) having filtering area 0.40″ diam., or use any suitable device having filtering area 0.40″ diam.

(2) *For off-bottom method.*—Single-unit type for intake of 1 pint on upstroke of plunger and discharge thru disk on down stroke, or 2-unit type, contg 1 unit for removal of 1 pint milk from bottom of can and another for filtering sample. Use sampling device long enough to permit reaching bottom of milk can, with filtering area $1\frac{1}{8}''$ diam.

(**b**) *Cotton sediment disks.*—Std lintine cotton disks or pads, $1\frac{1}{4}''$ diam., for use over flat wire screen in tester to expose filtration area $1\frac{1}{8}''$ diam. Disk must not contain phenolic resins or other chemicals that may contaminate milk.

Test sediment disks as follows: Filter 12 mg std sediment mixt. (60 ml aliquot (**d**)) thru pad, using clean flask to catch filtrate. Transfer filtrate to beaker, rinse flask 3 times with H_2O, and add rinsings to beaker. Filter filtrate thru 7 or 9 cm S&S White Ribbon paper (or equiv.) that has been washed with ca 200 ml H_2O, dried to constant wt at 100°, and cooled in covered dish in desiccator before weighing. Rinse beaker and paper thoroly with H_2O and dry to constant wt as above. Test at least 3 disks; av. wt sediment passing thru each disk should not be >2.8 mg. In addn, std disk prepd from fine mixt. should not appear to have sediment buried beneath surface.

(**c**) *Sediment filtering apparatus.*—App. must hold $1\frac{1}{4}''$ sediment disk and have effective filtering area $1\frac{1}{8}''$ diam. This $1\frac{1}{8}''$ area must be unobstructed except for wire screen or wire screen and perforated plate support for filter disk. App. should be supported in filter flask so vac. can be used for rapid filtration or flask air outlet closed to stop filtration. App. should have ca 80° funnel with min. capacity of 80 ml and max. capacity of 450 ml. Test app. by filtering H_2O suspension of C thru std disk. Disk should have clean, sharply defined border. When sediment suspension is filtered, sediment should be evenly distributed over disk with no pattern formation. Figs. 78 and 79 show suitable app.

(**d**) *Preparation of coarse standard sediment disks* (*1*).—Prep. uniform mixt. of oven-dried (100°) materials which meet following screening specifications. Grind all materials by hand with mortar and pestle.

	Per cent
Cow manure, thru No. 40	53
Cow manure, thru No. 20, retained on No. 40	2
Garden soil, thru No. 40	27
Charcoal, thru No. 40	14
Charcoal, thru No. 20, retained on No. 40	4

Place 2.00 g above mixt. in 100 ml vol. flask and moisten with 5 ml *1% Aerosol soln* or other suitable wetting agent, add 46 ml 0.75% gum soln such as algin soln, **36.002(aa)**, and bring level of liquid just into neck of flask by adding 50% (by wt) sucrose soln. Let stand at least 30 min., add few drops of alcohol, and dil. to vol. with the sucrose soln. Mix thoroly, pour into 250 ml beaker

FIG. 78.—SEDIMENT FILTERING APPARATUS, UNASSEMBLED

FIG. 79.—SEDIMENT FILTERING APPARATUS, ASSEMBLED

or 6–8 fl. oz. screw cap jar, and stir with mechanical stirrer at speed (ca 200–300 rpm) such that mixt. is thoroly agitated but very little air is whipped into suspension. Observe with light. Place blade of stirrer so that fine particles do not accumulate in small eddies at bottom of beaker.

Transfer, while stirring, 10 ml portion (200 mg std sediment) with large-tip graduated pipet to 1 L vol. flask, and dil. to mark with the 50% by wt sucrose soln. When thoroly mixed, each ml contains 0.2 mg sediment. Mix, pour into 1500 ml beaker, and stir with mechanical stirrer as above. If particles accumulate on side of beaker, wash down with portions of sediment suspension or push under with tip of pipet. While stirring, pipet definite vols of sediment mixt. and add to ¾ pint filtered sweet skim milk. Mix thoroly and pass mixt. thru std sediment disk in filtering app., (**c**). Pour milk gently down side of filtering app. and filter with very little or no suction. Wash container promptly with ¼ pint filtered skim milk. Let last portion of milk flow thru pad with no suction applied. If sediment does not appear to be evenly distributed over pad, add 15 or 20 ml skim milk and let it filter thru without suction. Repeat addn until sediment appears evenly distributed. Suck air thru disk ca 1 min. to remove excess skim milk.

For permanent record, mount and spray disks with 40% HCHO soln or with alc. soln contg 2.5 g each of menthol and thymol in 100 ml. Alternatively, if most of milk is removed by thoro aspiration, no preservative is needed. Dried pads may be coated with colorless plastic cement dild with 1–3 vols acetone so that mixt. is thin enough to pour easily. If acetone dissolves pigment from paper and stains pads, place pads on flat glass plate for treating with dild cement. Move pads while drying to prevent sticking to glass. When pads are almost dry, place light wt (*e.g.*, Petri dish) directly on them to prevent curling. Pads may be mounted with the plastic cement. (Std disks made from manure contg large amount of chlorophyll cannot be coated with plastic cement, as solvent exts chlorophyll and stains pad green. Use this method of preserving pads only if there

is no leaching of pigment from sediment on addn of dild plastic cement.)

Following above method, prep. series of disks contg sediment remaining from 0.0, 0.2, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0, 12.0, and 14.0 mg std mixt. Mark disks to show quantity of sediment (mg) used to prep. each pad. Do not use as std any pad on which sediment is not evenly distributed.

For comparison with tests on samples, entire series of disks may be used, but usually it is more convenient to select few disks denoting variations in grade that are applicable to particular investigations being made. If grading charts are prepd and reports made, indicate on chart and report whether mixed or off-bottom sample was used. If stds are to be handled or used for appreciable length of time, place them under glass, transparent plastic sheets, or other suitable materials. In using stds, grade sediment disk of sample as "more than __mg" or "less than __mg." When grading pads, disregard gross pieces of material (whole flies, hairs, large chunks of dirt or manure, etc.) but if such matter is present, list each separately on report.

(**e**) *Preparation of fine std sediment disks.*—Prep. mixt. from oven-dried (100°) ground cow manure, garden soil, and charcoal. Sift materials separately to pass No. 140 and be retained on No. 200 sieve as follows: Place max. of 100 g manure or soil and max. of 50 g charcoal on 8″ No. 140 sieve nested over No. 200 sieve. Add cover and receiver. Shake nested sieves by hand 5 min. at rate of ca 120 strokes/min. In max. batches of ca 20 g resift fractions remaining on No. 200 sieve as above 5 min. Use "on 200" fractions from second sieving and mix uniformly in following proportions: Cow manure 66%, garden soil 28%, and charcoal 6%. Proceed in as (**d**), beginning "Place 2.00 g above mixt. . . . " except to use H_2O instead of 50% sucrose for dilg the 10 ml aliquot to 1 L.

Where (**d**) states "While stirring, pipet definite vols . . . " proceed as follows: Det. approx. funnel capacity of filtering app., (**c**), by pouring H_2O into assembled app. with filter flask air outlet closed. Include H_2O that filters thru as part of funnel capacity. While stirring, pipet aliquots of sediment suspension into beakers. Add H_2O to make total vol. 20–50 ml less than funnel capacity, using min. total vol. of 60 ml and max. of 400 ml.

With filter flask air outlet closed to prevent filtration, mix dild aliquot and pour into app., (**c**), fitted with wet std disk, (**b**). Add 20–50 ml H_2O to beaker and rinse by swirling. Pour into funnel, keeping lip of beaker touching surface of H_2O if possible. (Rinse H_2O should nearly fill funnel if capacity is 450 ml or less.) Open flask air outlet. After H_2O has filtered thru pad, apply vac. and aspirate disk for ca 1 min. Remove pad and let dry in covered dish. If sediment is not evenly distributed, discard pad. After some practice, ca 75% of pads prepd should be acceptable. No preservative is required. Pads may be coated with dild plastic cement and used as in (**d**).

(**f**) *Photographic standards.*—Photographic stds (obtainable from American Public Health Association, 1790 Broadway, New York 19, N. Y.) may be used as guide in grading sediment pads, but it is preferable to use actual disks prepd as in (**d**) or (**e**). Stds that more nearly resemble disk being graded should be used in each case. Do not use photographs that have become faded, stained, soiled, or otherwise damaged.

36.010 CHECKING SEDIMENT TESTERS

To check sediment testing devices, proceed as follows: Measure actual quantity of milk delivered to assure that 1 pint is withdrawn and passes thru disk. Transfer 10 ml of the 2% sediment suspension in sucrose soln, **36.009**(**d**), using large-tip graduated pipet, to 10 gallons clean filtered H_2O in clean milk can. After thoroly agitating mixt. remove 1 pint with clean pint measure and filter thru $1\frac{1}{8}''$ diam. area of sediment disk, **36.009**(**b**), mounted on suitable funnel of correct size, *e.g.*, **36.009**(**c**). After thoro agitation of contents of milk can, again remove pint sample with the sediment testing device and pass thru sediment disk in exactly same manner as when testing milk. Repeat this operation with the tester several times to det. whether all disks so obtained give same sediment as disk obtained by filtering thru funnel, **36.009**(**c**).

36.011 COLLECTION OF SAMPLE (*2*)

(**a**) *Mixed sample method.*—For retail containers, 5 to 10 gallon cans, and storage tanks, use 1 pint or 1 gallon samples. Before mixing milk, transfer with small strainer any floating extraneous matter, such as flies, hairs, large chunks of debris, etc., to mounted disk, **36.012**(**a**), or mount on separate disk, properly identified. Mix milk thoroly in container before removing test portion. Avoid contamination of sample with foreign matter on stirrers or by any other means. For retail containers take 1 pint from mixed container or composite sufficient number to make 1 gallon Proceed as in **36.012**(**a**).

(**b**) *Off-bottom method.*—For 5 to 10 gallon cans take pint sample with either type of off-bottom tester from unstirred can of milk. Before withdrawing sample, remove with small strainer any floating extraneous matter as in (**a**). Take sample not $> \frac{1}{4}''$ off bottom of unstirred can of milk by inserting sampler and, during upstroke of plunger, drawing head of instrument once across diam. of can bottom or around circumference if

can has high center. Expel milk with gun in can and then with short stroke remove excess fluid from pad. Proceed as in **36.012(b)**.

36.012 DETERMINATION

(**a**) *Mixed samples.*—Pass sample thru properly adjusted disk, **36.009(b)**, held in correct position in tester. Warm 1-pint sample to 80–90°F and filter thru restricted area 0.40″ diam., **36.009(a)**(1). If single-unit off-bottom tester with special head is used, warm sample larger than 1 pint to 80–90°F and withdraw 1 pint with tester while stirring, or draw 1 pint into tester and warm milk by holding tester under running hot H_2O before discharging milk thru disk.

Warm 1 gallon sample to 80–90°F or filter cold thru $1\frac{1}{8}$″ diam. area of disk, **36.009(b)**. If milk is filtered at temp. <80°F, rinse disk by filtering ca $\frac{1}{2}$ pint sediment-free warm (90–100°F) H_2O thru disk before removing from tester. If milk is to be salvaged, do not dil. with H_2O. (Milk varies in its rate of flow thru disks; pasteurized milk may be more difficult to filter than raw milk. Other factors influencing rate of flow are temp., fat content, degree of clumping of fat globules, stage of lactation, presence of mastitic milk, and amount of sediment in sample.)

Remove disk from tester and mount on special sized paper or store in individual transparent waxed envelope. (If disk is placed on paper or in envelope while still moist, drying milk acts as adhesive.) Grade by comparison with the std disks, **36.009(d)** or (**e**), and indicate on report whether pad was graded wet or dry. (Character of sediment may be detd by microscopic examination.)

To prevent decomposition on storage, disk may be sprayed with HCHO soln or alc. menthol-thymol soln as in **36.009(d)**. Do not use glue to affix disk to paper; if disk becomes detached, moisten with few drops of H_2O and remount. Protect from contamination.

(**b**) *Off-bottom samples.*—Remove disk from tester, **36.011(b)**, and proceed as in (**a**), beginning "mount on special sized paper . . . "

36.013 Filth in Butter, Cheese, Cheese Products, Dried Milk Products, and Dairy Products in General

Use following methods independently or in various combinations. In all cases weigh 225 g into suitable container and use S&S ruled No. 8 paper for filtration. Cut hard cheese into small pieces.

(**a**) BUTTER.—FIRST ACTION

Place container in H_2O bath or oven at ca 80°. When fat seps, filter directly thru paper with suction, retaining most of curd and H_2O in container. After fat passes thru, filter remaining material. To facilitate filtration of curd, wash paper with near boiling H_2O during filtration. (For butter not filterable by this process, use (**c**).) Examine filter microscopically.

(**b**) EVAPORATED MILK, CONDENSED MILK, SWEET CREAM, SPRAY-DRIED WHOLE OR SKIM MILK.—FIRST ACTION

Reconstitute dried or concd products. Dil. reconstituted product with equal vol. hot H_2O, hot 3% $Na_2C_2O_4$ soln, or hot 2% Na_2CO_3 soln, and filter with suction. During filtration, wash paper continually with stream of near boiling H_2O to prevent accumulation of layer of particles which clog paper. Examine filter microscopically.

(**c**) BASIC METHOD FOR SOFT AND SEMI-SOFT CHEESE, AND SOUR CREAM; SOME DRIED WHOLE AND SKIM MILKS; AND BUTTER THAT CANNOT BE FILTERED BY (**a**).—FIRST ACTION

Heat sample in 1.5–2 L beaker with 800–1000 ml H_3PO_4 (1+40) with continuous stirring with slow speed mechanical stirrer until mixt. is boiling, or add cheese to boiling H_3PO_4 soln, and continue boiling up to 20 min. in order to disperse. Filter, without letting mixt. accumulate on paper, washing filter continually with stream of near boiling H_2O to prevent clogging. When filtration is impeded, add H_2O, dil. (1–5%) alkali, dil. H_3PO_4 soln, or hot alcohol until paper clears; then resume addn of sample suspension and H_2O. Examine filter microscopically.

(**d**) BASIC METHOD FOR HARD SKIM AND PART-SKIM MILK CHEESE (ROMANO, RICOTTA, FETA, PECORINO, SARDO, GOATS MILK CHEESE, SBRINZ, GOYA, WHEY CHEESE, ETC.).—FIRST ACTION

(1) Disperse sample as in (**c**), cool to 35–40°, and adjust to ca pH 7.5 with NaOH soln, then to pH 8 with Na_3PO_4 soln, and add filtered aq. ext. from 10 g pancreatin. Readjust to pH 8 after ca 5, 20, and 60 min. Digest at least 3 hr, preferably overnight. Adjust to pH 2 with H_3PO_4, bring to boil, boil ca 15 min., filter, and examine microscopically.

(2) If cheese does not filter, sep. into two ca equal portions. To each portion add ca 250 ml up to equal vol. alcohol, boil for addnl 15 min., filter, and examine microscopically.

(**e**) OPTIONAL METHOD FOR HARD SKIM AND PART-SKIM MILK CHEESE (ROMANO, RICOTTA, FETA, PECORINO, SARDO, GOATS MILK CHEESE, SBRINZ, GOYA, WHEY CHEESE, ETC.).—PROCEDURE

Add 400 ml H_2O to sample. Adjust to pH 8 with ca 10% soln of Adjusted Calgon, **36.002(f)**

Add filtered soln from 10 g pancreatin and 10 g Na_2SO_3, warm to 40°, and stir until thoroly mixed. Adjust to pH 8 with the Calgon soln at 15 min. intervals until stabilized. Digest overnight. Add equal vol. alcohol, stir, and adjust to pH 2 with HCl. Bring to boil on hot plate with constant gentle stirring. Boil 15 min. and filter. Keep paper clean with near boiling H_2O. In some cases, playing constant stream of hot H_2O on paper facilitates filtering. If some lumps of cheese remain in beaker, add acid-alcohol-H_2O soln (1 ml HCl/L 50% alcohol) and repeat boiling and filtering operations.

(**f**) OPTIONAL METHOD FOR HARD CHEESES.—FIRST ACTION

Cut or break 100 g sample into 2 L beaker and add 100 ml filtered aq. soln contg 20 g Calgon, **36.002**(**f**). Adjust pH to 8.5 with NH_4OH. Heat to 50° while stirring vigorously with mechanical stirrer until cheese is dispersed. (Time for dispersion at 50° depends on hardness of cheese.) Cool to 40°, adjust to pH 8, and maintain this pH thruout digestion. Add filtered soln from 20 g pancreatin, **36.002**(**t**), and let stand 2 hr at 40°. (Very hard, unripened, and whey cheeses may require overnight digestion.)

Adjust to pH 4 with HCl (1+19) and heat to 70° with vigorous stirring. (Unripened and whey cheeses contg little fat may be heated to 80°.) Continue stirring 15–60 min. until cheese is completely dispersed and filter. If paper clogs, add few ml HCl (1+19) followed by hot (ca 70°) H_2O. Examine papers microscopically.

(**g**) CHEESE CONTAINING MOLD, PLANT TISSUES, AND SPICES.—FIRST ACTION

Use (**c**), (**d**), or (**e**) to disperse cheese. Pour thru No. 140 sieve, washing thoroly with forcible stream of H_2O. Transfer material retained on sieve to beaker. Add 200 ml 2% H_3PO_4, boil until lumpy residue dissolves, and pour again thru No. 140 sieve, washing thoroly with forcible stream of hot H_2O. Transfer material on sieve with ca 200 ml 60% alcohol to trap flask and cool. Trap off, using gasoline and H_2O, filter, and examine microscopically.

36.014 Sediment in Cream, Butter, Cheese, Cheese Products, Dried Milk Products, and Dairy Products in General—First Action

(**a**) *Rapid method for sweet cream and cream in which curd is easy to disperse and in absence of mold.*—Place 1 pint sample in beaker or pan of convenient size, ca 2 L, and add ca 1 pint hot H_2O (70–90°). More or less H_2O may be added so that mixt. when ready for filtration is at 45–60°. Remove whole flies or other large filth particles which float to surface and which would be broken up by stirrer. Place these on sediment pad when completed. Place pan under malted milk stirrer, and add, while stirring, enough 40% Adjusted Calgon soln, **36.002**(**f**), to make mixt. slightly alk. to litmus. Use not <25 ml; excess will not interfere with filtration. Stir 30–60 sec. or until curd is broken up. Filter with vac. thru std sediment disk, **36.009**(**b**). If pad clogs, filter remaining portion thru fresh disk. Rinse pan and funnel with hot H_2O onto sediment disk.

(**b**) *Other dairy products.*—Proceed as in **36.013**(**c**), (**d**), (**e**), or (**f**), and filter thru std sediment disk, **36.009**(**b**). Violent mechanical agitation, such as is provided by malted milk stirrer, may be used to facilitate dispersion of product.

Compare with std sediment disks, **36.009**(**d**), (**e**), or (**f**).

36.015 Mold in Butter (*3*)—Official

(**a**) Carefully examine surface of sample and note any visible mold growth. To eliminate possibility of contamination by surface mold, scrape off and discard ⅛" of surface. Weigh, in 50 ml beaker, 1 g butter obtained from exposed surface. Add 7 g hot (50–60°) stabilizer soln, **36.002**(**aa**). Stir until mixt. is uniform and fat globules are 0.1–0.2 mm diam. Mount portion of mixt. on Howard cell, **36.001**(**j**)(**1**), and estimate mold as in **36.060.** Consider fields positive when single filament or combined length of 2 longest filaments exceeds ⅙ diam. of field.

(**b**) *Alternative procedure* (*staining*).—Add 1 or 2 drops crystal violet soln, **36.002**(**m**) to stabilizer-butter mixt. after butter is melted. Mix prepn thoroly and prep. slide as above.

NUTS AND NUT PRODUCTS

Shelled Nuts

36.016 Whole Nut Meats and Large Pieces—First Action

(**a**) *Light filth* (*except peanuts with adhering testa*).—Place 100 g sample in 1 L trap flask. Add 10 ml 10% Na oleate soln, **36.002**(**x**), and ca 600 ml 60% alcohol. Heat to boiling; then cool to <20°. Add 35 ml gasoline and ext. In first trapping let mixt. stand 5 min. before filling flask with 60% alcohol. Use 20 ml gasoline for second extn. Filter, and examine microscopically. Pour residue in trap flask onto No. 40 sieve. Rinse both flask and residue with H_2O. Transfer material on sieve to white enamel pan and examine for gross filth.

(**b**) *Light filth* (*peanuts with adhering testa*).—Place 100 g peanuts in 2 L trap flask. Add 250 ml Tween 80–60% alcohol soln, **36.002**(**bb**), mix well, and add 60% alcohol to total vol. of ca 800 ml. Trap off twice from 60% alcohol as usual, using 75 and 40 ml portions gasoline, resp. Stir occasionally during first 20 min. after flask is filled.

Let flask stand undisturbed addnl hr for each of the 2 extns. Trap off and filter, using 60% alcohol as rinse. (Acetone may be used to decolorize material on filter.) Examine filters microscopically.

36.017 Granulated Nut Meats or Mixtures Containing Substantial Amounts of Fine Granular Material—First Action

(**a**) *Heavy filth.*—Weigh 100 g sample into 600 ml beaker. Add ca 350 ml petr. ether and boil gently 30 min., adding petr. ether to maintain original vol. Decant solvent, taking care not to lose any coarse nut tissue, and discard. Add ca 300 ml $CHCl_3$ to beaker and let settle 10–15 min. Pour off floating nut meats and ca $\frac{2}{3}$ of the $CHCl_3$ thru 15 cm (or larger) paper in büchner, using care not to disturb residue in bottom of beaker. Repeat sepn with smaller quantities of mixt. of $CHCl_3$ and CCl_4 (1+1) until residue in beaker is relatively free of nut meat particles. Reserve residue on paper for (**b**). Transfer residue in beaker to ashless paper and examine for heavy filth. If appreciable amount of sand and soil is present, ignite paper in weighed crucible at ca 500° and weigh.

(**b**) *Light filth.*—Invert paper contg decanted nut tissue from (**a**) over smooth sheet of paper. Break up any caked material and dry overnight at room temp. or in oven 1 hr at ca 80°. Proceed as follows:

(1) *Granulated pecans.*—Transfer dried nut meats to heavy-wall 2 L trap flask. Add ca 300 ml 60% alcohol. Rinse down sides of trap flask and apply vac. ca 10 min. (Fit mouth of the heavy-wall flask with large rubber stopper with center hole fitted with glass tube of sufficient diam. to fit over protruding rod of trap flask. Control vac. with 2-way glass stopcock in vac. line. If stopcock tube is of sufficient diam. and length, it may be used as the tube thru center of stopper.) Swirl contents of flask occasionally to facilitate removal of entrapped air. Release vac. and trap off, using 50 and 25 ml portions gasoline for the 2 trappings, **36.003**(**a**). In first trapping, after stirring in gasoline, let stand 5 min. before filling flask with 60% alcohol. To trappings add HCl equal to ca 1% of the vol., bring to boil, filter, and examine microscopically.

(2) *Granulated black walnuts.*—Transfer dried nut meats to 2 L trap flask, assisting transfer and rinsing the 15 cm paper with ca 300 ml 60% alcohol. Stir slightly and let mixt. soak 10 min. Add 250 ml Tween 80–60% alcohol soln, **36.002**(**bb**), and mix. Add quickly 250 ml Na_4EDTA-60% alcohol soln, **36.002**(**dd**), and ca 70 ml gasoline. Stir immediately as usual. Fill flask with 60% alcohol. (Add reagents, mix gasoline, and fill flask with 60% alcohol without interruption. Operate only 1 flask at time for these 3 steps.) After flask is filled, stir occasionally during first 20 min.; then let stand undisturbed addnl hr. Trap off, taking care not to disturb interface. Rinse rod and neck of flask with 60% alcohol and filter. If debris is present in trappings, add HCl equal to ca 1% of the vol., bring to boil, and filter. Repeat extn, using 40 ml gasoline and 1.5 hr standing. Examine filters microscopically.

(3) *Granulated nut meats (except pecans and black walnuts).*—Proceed as in (2) thru addn of the 60% alcohol. Instead of letting mixt. soak 10 min., heat to incipient boil. Do not boil or filth recovery will be lowered. Cool to $<20°$ and proceed as in (2), beginning "Add 250 ml Tween 80–60% alcohol . . ."

Shredded Coconut

36.018 Filth—First Action

(**a**) *Heavy filth.*—Proceed as in **36.028**, using 100 g sample in 400 ml beaker.

(**b**) *Light filth.*—Weigh 100 g sample into 2 L beaker. Add 1 L hot 5% borax soln, boil 10–15 min., pour thru 8″ No. 140 sieve, and wash well with hot H_2O. Using wide aperture funnel, transfer coconut to 2 L trap flask with ca 700 ml 60% alcohol. Wash sieve with forcible stream of hot H_2O, collecting final residue at one edge of screen and transferring to trap flask with stream of 60% alcohol. Add 50 ml gasoline to trap flask and mix thoroly. Fill flask with 60% alcohol. Let stand 30 min. with occasional gentle beating and lifting of coconut material to free any rodent hairs adhering to it. Trap off gasoline layer, using 60% alcohol as rinse, and filter thru ruled paper. Add 25 ml gasoline to trap flask and make second extn. After 30 min., trap off, and filter on second paper. Examine filters microscopically.

Peanut Butter

36.019 Preparation of Sample—First Action

Examine individually at least 3, and preferably 6, jars. If jars contain <1 lb each, make 3–6 composites of at least 2 jars each so that composite samples will be ca 1 lb. Remove contents of each jar and mix thoroly, preferably in evapg dishes of convenient size, using heavy table fork or spatula. Peanut butter after warming may also be mixed in the jar by means of mixer, **36.001**(**c**), equipped with stiff paddles. If large number of jars is to be examined, make composite samples by thoroly mixing contents of 3–6 jars of equal size.

36.020 Water-Insoluble Inorganic Residue ("WIIR") and Excreta—First Action

Weigh 100 g sample into 250 ml beaker (hooked-lip type), add ca 10 ml petr. ether, and mix thoroly. Continue to add petr. ether, mixing

thoroly until ca 150 ml has been added. Cover, let settle 25 min., and decant 100 ml of the petr. ether layer and floating light tissue, taking care not to lose any coarse peanut tissue. Add ca 125 ml petr. ether to residue and mix, let settle 15 min., and decant 100 ml as before. Repeat with third ca 125 ml addn of petr. ether, stir, wash down sides of beaker with stream of petr. ether, let settle 10 min., and decant 100 ml. Discard all decanted portions of petr. ether.

Evap. remainder of petr. ether from residue in beaker; gentle heat may be used. Add 150 ml $CHCl_3$ to residue and mix thoroly; cover beaker and let settle 20 min. Stir top layer several times during this period. Decant $CHCl_3$ and floating peanut tissue onto 15 cm paper in büchner, being careful not to disturb heavy residue in bottom of beaker. Save all decanted peanut tissue for detn of light filth, **36.021.**

Repeat extn with small quantities of $CHCl_3$, rinsing all particles from sides of beaker. At this point watch for fragments of rodent excreta pellets on top of NaCl in bottom of beaker; do not decant them. (If sample contains considerable peanut skin, it may be necessary to use mixt. of $CHCl_3$ and just enough CCl_4 to float skin particles away from heavy residue of NaCl, sand, etc.) Dry residue in air.

Add 50 ml HCl (1+35) to residue in beaker; then add 90 ml boiling H_2O and let stand 30 min. with occasional stirring to dissolve any phosphate, carbonate, or anhydrite ($CaSO_4$) included with the NaCl. Decant liquid thru ashless filter in 60° glass funnel and finally transfer residue with hot H_2O. Test filtrate for sulfate by adding 5 ml satd $BaCl_2$ soln. Wash residue on filter several times with hot H_2O.

If test for sulfate in filtrate was positive, test residue on filter by placing clean beaker or test tube under funnel and treating residue with 25 ml HCl (1+35), adding little at time. Test filtrate with 20 drops satd $BaCl_2$ soln (fine white ppt of $BaSO_4$ indicates presence of anhydrite in residue on filter; allow 5 min. for ppt to appear). Wash residue on filter with hot H_2O until all HCl is removed.

Examine residue microscopically for fragments of rodent excreta pellets (identified by presence of rodent hair fragments in mass), insect excreta pellets, and other filth. Ignite paper in weighed crucible over medium Bunsen flame or in muffle furnace at ca 500°. Cool, and weigh crucible and contents to nearest 0.5 mg. If "WIIR" is excessive and application of above test indicates that all $CaSO_4$ has not been removed, make quant. detn of either Ca or sulfate in "WIIR" in crucible, as in **31.060** or **31.059**. Calc. this wt to $CaSO_4$ and correct wt of "WIIR."

36.021 Light Filth—First Action

Invert, over smooth sheet of paper, the 15 cm filter paper from büchner, **36.020**, contg decanted peanut tissue. Retain paper for rinsing later. Break up any caked or lumpy peanut material and dry overnight at room temp. or in oven 1 hr at ca 80°. Transfer dry residue to 600 ml beaker. Rinse paper with H_2O, adding washings to beaker. Add 300–400 ml H_2O and stir until smooth. Add filtered aq. ext. from 5 g pancreatin, **36.002(t)**, and mix. Adjust to pH 8 with Na_3PO_4 soln. Readjust pH after ca 15 min. and again after ca 45 min. Add 5 drops HCHO and digest overnight at 37–40°. Cool, transfer digested material to 2 L trap flask, and dil. with H_2O to 800–900 ml. Trap off twice with 35 and 25 ml gasoline, resp., **36.003**(a). Filter, and examine microscopically.

36.022 Rocks and Decomposed Peanuts in Coarse Peanut Butter —First Action

Remove entire contents of jar to 1500 ml beaker or other suitable container. Add ca 700 ml CCl_4 and mix thoroly, using mixer, **36.001(c)**, if convenient. Rinse jar with CCl_4 and add rinsings to beaker. Let mixt. stand at least 15 min. with occasional stirring. Decant ca ⅔ of mixt. and add ca 200 ml CCl_4. Let stand 5 min. and decant. Wash down sides of beaker with CCl_4 and repeat decantation until residue is free from peanut tissue. Save all decanted material.

Dry residue in beaker and wash out salt with hot H_2O. If large quantity of sand is present, wash residue to remove salt, phosphate, carbonate, and anhydrite as in **36.020**, fourth par. Transfer residue to ashless filter paper and examine under low-power microscope. Report number and approx. size of rocks and other extraneous material. If much sand is present, ignite filter and weigh residue, including rocks, reporting result in mg/100 g peanut butter.

Pour decanted CCl_4 peanut mixt. thru No. 14 sieve and examine residue for gross filth, stems, other extraneous material, and decomposed peanut tissue.

36.023 Glass —Procedure

Take precautions to avoid picking up glass particles when removing contents from glass container and during analysis. Use only stainless steel, aluminum, plastic, or other nonglass laboratory ware. Filter reagents.

Loosen jar tops and warm jars of peanut butter several hrs at ca 50° in oven.

Add 10 g household detergent powder, such as alkyl aryl sulfonate, to 2 L ca 70° H_2O. Mix

thoroly and filter thru folded filter into 3–4 L stainless steel beaker. Place beaker in ca 70° H_2O bath and set beaker with bath under paddle type stirrer, **36.001(c)**. While stirring detergent soln, add entire contents of 12 oz jars, or composite contents of two 6 oz jars, or subdivided 12 oz sample portions of larger containers. Rinse jar and top portionwise with ca 100 ml kerosene and finally with detergent H_2O, and add washings to mixt. Continue stirring ca 3 min. Stop stirrer, scrape bottom and sides of beaker with metal spatula, and mix manually any residual peanut butter. Replace paddle and stir again ca 3 min. until peanut material is completely dispersed. Stop stirrer, rinse paddle blades over beaker with H_2O, and promptly begin decantation procedure.

In decanting, let mixt. settle full 15 sec. each time after beaker is refilled with 55–70° H_2O, with beaker in upright position, or leaning up to 45°. Pour off *smoothly*, after indicated standing period, not >80% of beaker contents. Repeat as necessary until coarser particulate matter with small amount of clear H_2O is left. Pour off carefully as much of this clear H_2O as possible, still leaving particulate matter in beaker. Rinse down sides of beaker thoroly with alcohol from plastic wash bottle, and continue decantations after standing 15 sec. as above, finally pouring off all possible alcohol without losing particles. Rinse sides of beaker with ca 250 ml $CHCl_3$. If plant particles tend to aggregate, add more alcohol down sides of beaker until particles are freely mobile. After 20 sec. standing, decant, agitating plant particles to free any mechanically entrapped glass. Be careful that glass from beaker pocket is not picked up by plant material as it sweeps forward from "back" side of beaker during decantation. Remove as much plant material as practical by $CHCl_3$ decantations, after 20 sec. standing, with preceding precautions. Transfer beaker contents to filter paper (preferably black) on büchner. Invert beaker over filter and scrupulously rinse sides and bottom of beaker with jet streams of alcohol and warm H_2O alternately from plastic wash bottles.

Examine filter microscopically at 30× for glass particles, using only H_2O as moistening agent.

CEREAL FOODS

Baked Products, Cereals, and Alimentary Pastes

36.024 Insect Fragments and Rodent Hairs—First Action

(**a**) *Sieving*.—Weigh 225 g sample into 2 L beaker, add enough hot H_2O to soften and sat. material, and proceed as in (1). If lumps persist or if H_2O is not immediately absorbed uniformly thru entire mass (*e.g.*, in case of hard English-type cookies), proceed as in (2).

(1) Adjust mixt. to pH 7–8 with ca 5% Na_3PO_4 soln. Stir and break up material as much as possible. Cool to 40° and add 100 ml pancreatin soln, **36.002(t)**. Stir thoroly and readjust to pH 7–8. Let stand 30 min., stir, and readjust pH.

(2) Estimate vol. mixt. and add HCl to ca 1+49 concn. Boil until solids become finely divided and so digested that mixt. will not froth over when covered during boiling. Neutralize to ca pH 6 with NaOH soln; then add Na_3PO_4 soln to pH 8 and continue as in (1)

For white flour products, add 2 ml HCHO and digest overnight. For products made from whole wheat and rye flours and from similar materials of high bran content, digest only 2–3 hr.

Pour digested material thru 5″ or 8″ No. 140 screen. While pouring, play forcible stream of hot H_2O from tap on this material. Wash well with large stream of hot H_2O. After complete washing (no starchy material visible unattached to bran), wash twice alternately with alcohol and $CHCl_3$ in that order, and then rinse thoroly with alcohol and finally with H_2O.

Transfer material to filter paper if little residue remains or to 1 or 2 L trap flask if large amount remains. Transfer bulk of material with spoon. Rinse residue from screen with 60% alcohol from wash bottle. Wash screen with forcible stream of hot H_2O, collecting final residue at one edge of screen and transferring to trap flask with stream of 60% alcohol as above. Add 400 or 900 ml 60% alcohol, depending on size of trap flask.

Boil 20 min. Cool to <20° and add 20 or 40 ml gasoline; fill flask with 60% alcohol, and trap off as usual. Trap off second time. Use care in stirring and during addn of alcohol to prevent formation of emulsion or inclusion of air. If residue in flask tends to rise, stir material down 2 or 3 times. Filter trapped-off material and examine microscopically.

(**b**) *Direct trapping*.—To 1 L boiling HCl (1+49) add 225 g sample, and continue heating 30–40 min., or until mixt. becomes finely divided mass that will not froth over when covered. Cool somewhat, partially neutralize with NaOH soln, adjust to pH 7–8 with Na_3PO_4 soln, cool to 35–40°, and digest with pancreatin as in (**a**). Bring to boil, cool, transfer to 2 L trap flask, ext., and examine microscopically.

Flours (White, Wheat, and Corn)

36.025 Insect and Rodent Filth—First Action

(**a**) *Light filth*.—Weigh 50 g flour into 600 ml beaker; stir into smooth slurry with 50 ml

pancreatin soln, **36.002**(t), dild with 100 ml H_2O. Dil. with H_2O to total vol. of ca 400 ml, and adjust to pH 8 with Na_3PO_4 soln. Readjust pH after ca 15 min. and again in ca 45 min. Add, with stirring, 3 drops HCHO soln and digest 16–18 hr at room temp. or not >40°. Transfer to 2 L trap flask and ext. as in **36.003**(**a**). Stir 1 min. and use deodorized kerosene and H_2O as solvents. Combine trappings and rinsings in beaker, transfer to 2 L trap flask, and trap off as above. If considerable starchy material is in ext., hydrolyze with HCl as in **36.003**(**b**). Examine papers microscopically.

(**b**) *Rodent excreta.*—Proceed as in **36.028.**

36.026 Insect Eggs (*4*)—**First Action**

Transfer 50 g flour to No. 100 sieve (if >ca 0.1 g residue is obtained, No. 60 or No. 80 sieve should be used to prevent slow filtration after digestion) and sift gently until no more flour passes thru. Transfer portion of residue remaining on sieve to 250 ml beaker and wet with 2–3 ml alcohol. Add 30 ml H_2SO_4 (1+19), cover beaker, and heat on steam bath 10 min. Filter thru paper on suction funnel, using min. suction necessary to filter. Keep beaker partially inverted over funnel and rinse with H_2O. Turn off suction. Add 15–20 ml ca 0.1*N* I to paper in funnel. Allow 10–15 sec. for I to stain contents. Apply gentle suction. After I passes thru filter, wash paper with 25–30 ml 1% H_2SO_4, followed by several small H_2O washes. Transfer paper to Petri dish and examine at once under 20× magnification.

36.027 Insect Excreta (*5*)—**First Action**

(**a**) *Optional for 1–4 samples.*—Weigh 0.20 g flour on weighed flat glass disk 7–7.5 cm diam. Add clove oil and spread mixt. into thin uniform layer. (Enough oil should be present to clear flour and present smooth surface of oil, but not so much that mixt. flows off disk.) Place wire grid over disk and examine microscopically with dark background and intense reflected light. Depending upon size of plate, larger quantities of flour and ruled glass plate can be used and oil-flour mount covered with glass, *e.g.*, use 0.5 g flour on tomato rot count plate, **36.001**(**m**). Weigh flour in counterbalanced scoop or directly on plate. Thoroly sat. flour on counting plate, cover with glass, and count insect excreta. To move or turn suspected particles, gently apply pressure or move cover slightly while observing thru microscope.

(**b**) *Optional in multiple-sample schedule.*—Tare 2–8 small numbered vials on each balance pan and weigh by shifting weights from one side to other. (If desired, larger portion may be weighed in beaker and some of flour floated off in $CHCl_3$-ether or $CHCl_3$-toluene mixt., sp. gr. 1.40, before transferring to filter paper.) Rinse contents of each vial onto smooth-surface, ruled paper in Hirsch funnel with $CHCl_3$ or CCl_4. Transfer paper to Petri dish, flood with clove oil, and examine with dark background and intense reflected light.

Whole and Degerminated Corn Meal, Corn Grits, Rye Meal, Wheat Meal, Whole Wheat Flour, Farina, and Semolina

36.028 Rodent Excreta (*6*)—**Official**

Weigh 50 g sample in 250 ml hooked-lip beaker. Add $CHCl_3$ to within ca 1 cm of top, mix thoroly, and let settle at least 30 min., stirring surface layer occasionally. Decant $CHCl_3$ and floating tissue onto büchner, taking care not to disturb heavy residue in bottom of beaker. Before decanting, take care that floating layer has not become so compact as to render this operation difficult. Add quantity of CCl_4 equal to quantity of $CHCl_3$ and tissue left in beaker, let settle again, and decant as before. Repeat this process with mixt. of equal parts $CHCl_3$ and CCl_4 until very little tissue remains in beaker. Take care not to decant any rodent excreta fragments that may be present. Wash residue in beaker onto 7 cm ruled paper with stream of $CHCl_3$ or CCl_4 and examine microscopically.

36.029 Insects, Insect Parts, and Rodent Hairs—First Action

Draw air thru material in büchner, **36.028,** until liquid evaps. Air dry overnight or dry in oven 1 hr at ca 80°. (CAUTION: In oven drying, phosgene is liberated and adequate ventilation must be provided.) Transfer residue to 1 L trap flask. Add 100 ml 60% isopropyl alcohol, previously satd with gasoline, **36.002**(**q**), and mix thoroly. Wash down sides of flask with the alcohol-gasoline soln until ca 400 ml is added, and soak 30 min. Trap off twice, using 20–30 ml gasoline for each trapping and the 60% isopropyl alcohol satd with gasoline as flotation medium. In first trapping, let stand 5 min. after stirring in gasoline before filling flask. Filter and examine both trappings microscopically.

Rye Flour

36.030 Insects, Insect Parts, and Rodent Hairs—First Action

Weigh 50 g flour into 2 L trap flask, add 300 ml 60% alcohol, mix, and let stand 10 min. Add 250 ml Tween 80–60% alcohol soln, **36.002**(**bb**), and mix. Quickly add 250 ml Na_4EDTA-60% alcohol soln, **36.002**(**dd**), and ca 70 ml gasoline. Stir immediately 1 min. in usual manner. Fill flask with 60% alcohol. (Add reagents, mix gasoline, and fill flask with 60% alcohol without interruption. Operate only 1 flask at time for these

3 steps.) Stir occasionally during first 20 min. after flask is filled.

After 20 min., rotate plunger to remove flour which settled on top surface. Raise rod so that plunger is above mass of flour at bottom of flask. Clamp unrinsed rod in place (clothes pin is convenient) so that plunger is held above flour mass to minimize flour settling on it. Let flask stand *undisturbed* addnl hr. Trap off, taking care not to disturb interface, rinse neck of flask with 60% alcohol, and filter. Repeat extn, using 40 ml gasoline and 1.5 hr standing. Examine filters microscopically.

Cereal Grains

36.031 Internal Insect Infestation—First Action

Mix grain by passing 6 times thru Jones sampler, recombining sepns before each pass. Sep. slightly >100 g and weigh 100 g. Transfer weighed sample, small amount at time, to 5″ or 8″ No. 12 sieve, and with stiff bristle brush, work insects thru sieve as completely as possible.

Grind screened sample in Labconco mill (Laboratory Construction Co., Kansas City, Mo., or equiv.) set at 0.061″. (Dry damp or tempered grain in force-draft oven 1 hr at 70–80° or 2 hr in oven without draft.) Transfer cracked grain, including any residue in mill, to 2 L trap flask, trap as in **36.003**(**a**), using 60% isopropyl alcohol satd with gasoline, **36.002**(**q**), and gasoline as solvents, and filter on 10XX bolting cloth, **36.001**(**b**). If considerable starchy material is in ext., hydrolyze with HCl as in **36.003**(**b**). Examine as in **36.003**(**g**) except use 15× as lower limit of magnification. Count only whole insects, insect heads, cast skins, and head capsules.

Starch

36.032 Filth—First Action

Weigh 225 g starch into 1500 ml beaker. Add, with stirring, 1200 ml cold H_2O (15–20°). Stir out lumps and pour thru 5–8″ No. 140 sieve. Wash with cold running H_2O. Rinse particles from sieve onto filter paper, first using H_2O and then 60% alcohol. Examine paper microscopically.

Brewer's Grits

36.033 Rodent Excreta—Procedure—*See* 36.028

36.034 Insect Fragments and Rodent Hairs—First Action—*See* 36.029

Soy Bean Flour

36.035 Light Filth—Procedure

Weigh 50 g sample into 600 ml beaker, add 150 ml petr. ether, and mix thoroly. Let settle 5 min. and decant ca 100 ml of the solvent and any floating light tissue, avoiding loss of any heavier flour materials. Replenish petr. ether and mix with residue. Let settle 5 min. and decant as before. Discard decanted portions. Evap. petr. ether from residue with gentle heat, stirring as residue dries to avoid formation of lumps. Dry until no petr. ether remains and proceed as in **36.025**(**a**), beginning "stir into smooth slurry . . . "

Popped Popcorn

36.036 Filth—Procedure

Weigh 50 g corn into 2 L trap flask. Add 500 ml hot H_2O, boil 15 min., and cool to room temp. Add 35 ml gasoline, mix, and let stand 5 min. Fill with H_2O, trap off, filter, and examine microscopically.

Unpopped Popcorn, Cereal Grains, Peas, Beans, Etc.

36.037 External Contamination—Procedure

Transfer 225 g sample to 2 L trap flask. Add 600 ml 40% alcohol and boil gently, with frequent stirring, 5 min. Cool, trap off, using gasoline and 40% alcohol, filter, and examine microscopically.

EGGS AND EGG PRODUCTS (*7*)—FIRST ACTION

(Eggs may be contaminated with chicken excrement, dirt, sand, metal fragments, hairs, and feathers, depending upon condition of the eggs, method of manufacture, and storage conditions. Method of isolation of contaminants depends upon nature of product (whole, whites, or yolks) and physical state (fresh, frozen, or dried).)

36.038 Special Reagents

(**a**) *Arsenophosphotungstic acid soln.*—Dissolve 25 g $Na_2WO_4.2H_2O$ in 150 ml H_2O and add 12.5 g As_2O_5, 6.2 ml H_3PO_4, and 5 ml HCl. Boil mixt. 20 min., cool, and dil. to 250 ml with H_2O.

(**b**) *Igepon TK 32*®.—Surfactant available from Antara Chemicals, 435 Hudson St., New York, N.Y. Filter before use.

(**c**) *Phenolphthalein soln.*—Prep. 5% soln in alcohol, dil. with equal vol. H_2O, and filter.

(**d**) *Protease "F"*®.—Disperse 2.5 g crude enzyme (available from Takamine Labs., Clifton, N. J.) with few ml H_2O in malted milk-type stirrer. Dil. to ca 250 ml, mix 30 min., centrifuge, and filter.

(**e**) *Disodium phosphate soln.*—Filtered satd soln (ca 100 g anhyd. salt/L), and filtered 6% (anhyd. basis) aq. soln.

(**f**) *Trisodium phosphate soln.* Filtered satd soln.

(g) *Versene ® soln.*—10% filtered aq. soln of tetra Na salt of ethylenediaminetetraacetic acid.

36.039 Insect and Rodent Filth and Foreign Matter

(a) *Frozen whole eggs or yolks.*—Weigh 100 g sample, thawed at room temp., into wide-mouth centrifuge bottle. Add 30 ml 6% Na_2HPO_4 soln and stir. Shake vigorously ca 2 min., add 30–40 ml more 6% Na_2HPO_4 soln, and shake vigorously 1–2 min. Dil. with 6% Na_2HPO_4 soln to fill bottle and centrifuge whole eggs 5 min. at 1500 rpm or yolks 5 min. at 800 rpm. Decant ca $\frac{3}{4}$ liquid into 1.5 L beaker. Add ca equal vol. 6% Na_2HPO_4 soln to residue, mix well, centrifuge as above, and decant closely, adding supernatant to decanted liquid. Retain sediment for isolation of uric acid, **36.040**(a).

To combined supernatants add, with thoro stirring, mixt. of 200 ml pancreatin soln, **36.002**(**t**), and 12 ml phthln soln, **36.038**(**c**). Add 16 ml Versene soln, **36.038**(g), stirring thoroly. Let stand 10 min., stirring occasionally, and adjust to pH 8, using indicator paper. Add 2 ml Igepon and transfer beaker to 37–38° H_2O bath. Readjust to pH 8 at 15, 30, and 60 min. and then at hourly intervals until placed in incubator at ca 37° overnight. Readjust to pH 8 and filter, rinsing paper occasionally with hot tap H_2O. Examine while paper is still moist.

(b) *Liquid or frozen egg whites.*—Weigh 100 g sample (thawed at room temp.) into 1 L beaker and add 375 ml 37–38° H_2O and 5 ml Versene soln, **36.038**(g), with thoro stirring. Let stand 10 min., stirring occasionally. Add 6 ml phthln soln, **36.038**(**c**), and adjust to pH 8 with H_3PO_4 (1+9), using indicator paper. Add 100 ml pancreatin soln, **36.002**(**t**), and 5 ml Versene soln, **36.038**(g), and readjust to pH 8. Place in 37–38° H_2O bath, stirring at 15 min. intervals. Readjust to pH 8 at 15, 30, and 60 min. After 2 hr, add 2 ml Igepon, readjust to pH 8, and hold at ca 37° until digestion appears complete, overnight if necessary. Filter and examine microscopically. Check amorphous white material for uric acid as in **36.041**.

(c) *Dried whole eggs.*—Add, with thoro stirring, 25 g sample to 600 ml beaker contg 100 ml H_2O and 10 ml alcohol. Add 200 ml pancreatin soln, **36.002**(**t**), and 12 ml Versene soln, **36.038**(g), with thoro stirring. Let stand 10 min., stirring occasionally; then adjust to pH 8 with satd Na_3PO_4 soln, using indicator paper. Place in 37–38° H_2O bath, and stir and adjust pH at 15 min. intervals until bath temp. is reached. Hold at 37°, adjusting pH at hourly intervals during 4–5 hr, and then digest overnight in incubator. Readjust to pH 8, and filter while warm. Examine microscopically, keeping paper moist. Check amorphous white material for uric acid as in **36.041**.

(d) *Dried egg whites.*—Weigh 25 g sample into 600 ml beaker. Dil. 4.5 ml Igepon to 35 ml with H_2O and add 5 ml of dild soln to beaker, rotating and shaking beaker until sample absorbs liquid. Continue adding Igepon soln in same manner until 35 ml is used. Stir to smooth slurry and let stand 10 min., stirring occasionally. (Material must be finely dispersed at this point.)

Add slowly, with thoro stirring, 20 ml H_2O. Continue stirring while adding 5 ml Versene soln, **36.038**(g), and 2.5 ml phenol red, **13.022**. After 5 min. neutralize with few drops H_3PO_4 (1+9). Adjust to faint pink with satd Na_2HPO_4 soln which has been made neutral to phthln. Add 250 ml Protease F soln prepd immediately before use and adjust to pH 6.0–6.5, using pH meter. Place in 37–38° H_2O bath and stir frequently during 1 hr. Readjust pH, using meter, at 20, 40, and 60 min. and then at hourly intervals during 3–4 hr. Scrub down sides of beaker, wash down sides with 50–100 ml ca 37° H_2O, and place in incubator at 37° overnight. Scrub down sides of beaker, readjust pH, add ca 100 ml ca 37° H_2O, and digest ca 1 hr longer. Filter and examine microscopically, keeping paper moist. Check amorphous white material for uric acid as in **36.041**.

(e) *Dried egg yolk.*—Weigh 25 g sample into 150 ml tall-form beaker, add 50 ml petr. ether, and stir well. Continue stirring while adding solvent to almost fill beaker and then stir top 1 min. Let stand and decant petr. ether into 250 or 400 ml beaker. Repeat solvent treatment twice and filter composited petr. ether thru ruled paper. Air-dry paper thoroly and reserve for pancreatin digestion. Discard petr. ether.

Remove solvent completely from residue in original 150 ml beaker on steam bath. (Continuous stirring may be required to prevent bumping.) Add to dried residue satd Na_2HPO_4 soln in small portions with stirring until beaker is ca $\frac{2}{3}$ full. (Material should be in form of smooth, finely-divided suspension.) Transfer to 600 ml beaker, rinsing with ca 100 ml satd Na_2HPO_4 soln. Add 5 ml phthln soln, **36.038**(**c**); if strong red color develops, discharge immediately with H_3PO_4 (1+9). Adjust to pH 8 with satd Na_3PO_4 soln, using indicator paper.

Add 100 ml pancreatin soln, **36.002**(**t**), to suspension and add reserved material from paper with spatula. Adjust to pH 8 and place in 37–38° H_2O bath 2 hr. Adjust pH at 20 and 60 min. After 2 hr, scrub down sides of beaker, adjust to pH 8, and wash down sides of beaker with ca 200 ml ca 37° H_2O. Hold at 37°, adjusting pH at hourly intervals, and incubate at ca 37° overnight.

Readjust pH and let stand 10 min. Without stirring, decant onto filter paper, using full suction, washing paper occasionally with warm tap H_2O. Wash paper well with warm tap H_2O. Ex-

amine microscopically, keeping paper moist. Check amorphous white material for uric acid as in **36.041**.

36.040 Sedimentation Method for Chicken Excrement and Heavy Filth

(a) *Frozen whole eggs or yolks.*—To sediment in centrifuge bottle, **36.039(a)**, add ca 0.5 vol. H_3PO_4, and warm on steam bath. Transfer to 250 ml beaker, boil 3–5 min., and filter while boiling. Examine at 30× for metal and glass fragments, and chicken excrement. Check amorphous white material for uric acid as in **36.041**.

(b) *Dried egg yolk.*—Add 25 g sample in small portions with continuous stirring to mixt. of 75 ml H_3PO_4 (1+9) and 5 ml Igepon in 150 ml tall-form beaker. Stir to smooth paste and add H_3PO_4, few ml at time, to fill beaker while stirring. Stir top layer 1 min. and let stand 5 min. Decant ca ⅔ vol. into 250 ml beaker and add H_3PO_4 (1+9) to both beakers equal to vol. present. Stir contents of both beakers 1 min. and let stand 5 min. Again stir top layers 1 min. and slowly add H_3PO_4 with stirring to fill both beakers. Let stand 5 min. and repeat stirring and standing. Decant both beakers closely into 1 L beaker.

Dil. material in 1 L beaker with H_2O, stirring continuously, until full. Stir top layer 1 min. and let stand 5 min. and repeat stirring and standing. Decant closely, discarding supernatants. Composite all residues in 250 ml beaker by transferring with H_3PO_4 (1+9) from wash bottle. Decant acid and floating egg material and transfer residue to ruled paper with H_2O, using min. suction. Wash residue with two 30 ml portions H_2O, using min. suction. Examine microscopically, keeping paper moist. Check amorphous white material for uric acid as in **36.041**.

36.041 Test for Uric Acid

Transfer white amorphous grainy particles to white spot plate. (Do not use metal instruments.) Add 1 drop 1% NaOH soln and macerate with small glass rod. Add 1 drop 15% NaCN soln and 1 drop arsenophosphotungstic acid soln, **36.038(a)**. Prompt development of blue color indicates presence of uric acid or its salts. Perform blank on reagents.

POULTRY, MEAT, AND FISH PRODUCTS

36.042 Filth and Sand in Chicken Giblet Paste—Procedure

Weigh 100 g sample into 400 ml lipped beaker. Add $CHCl_3$ to within 1″ of top of beaker, mix thoroly, and let settle ca 30 min., stirring top layer occasionally. Make opening in floating layer and decant most of $CHCl_3$. Add, stir, and decant $CHCl_3$ 3 times. Retain decanted $CHCl_3$ and filter thru 10XX bolting cloth. Examine microscopically. After third extn, decant paste layer into 2 L trap flask. Evap. most of $CHCL_3$ from material in flask.

Add ca 50 ml hot H_2O to residue in beaker. Decant and wash residue thru ashless filter in 60° funnel. Wash with warm H_2O. Transfer filter to Petri dish and examine for rodent excreta, sand, etc. To weigh sand, etc., fold paper, dry in weighed crucible, ignite, and weigh to nearest 0.5 mg.

To paste in trap flask add 200 ml hot H_2O and heat on steam bath 15 min. Cool to room temp. Add 35 ml gasoline, mix, and let stand 10 min., stirring several times. Trap off with H_2O, filter, and examine microscopically.

36.043 Filth in Canned Fish—Procedure

If can contains 1 lb or less, transfer total contents to 2 L trap flask, **36.001(o)**. Reduce larger portions to 1 lb. Avoid breaking fish into small particles. Cover fish with hot H_2O and rinse can and lid with 25 ml kerosene, letting rinsings enter trap flask. Mix, and fill flask with warm H_2O. Trap off, filter, and examine. Trap off second time, using 20 ml kerosene, filter, and examine microscopically.

36.044 Glass in Meat Scraps—Procedure

Weigh 50 g well-mixed sample into 400 ml beaker, add ca 350 ml CCl_4, and mix contents thoroly. Let stand, with occasional stirring, 30 min. Decant and discard CCl_4 and floating org. matter, leaving bone and heavy matter in beaker. Add more solvent and again decant if necessary. Wash out adhering CCl_4 with one rinse of ca 100 ml alcohol and then one rinse of 350 ml H_2O, decanting slowly after each addn of alcohol or H_2O to prevent loss of heavy matter. Add 50 ml HCl and heat on steam bath ca 1 hr.

Wash residue with 3–6 portions of H_2O, using 350 ml each time; rinse once with ca 50 ml alcohol and then 2 or 3 times with CCl_4; decant after each addn and finish with rinse of $CHCl_3$. Let heavy residue dry thoroly and weigh to nearest mg. Pass dry residue thru No. 40 and No. 60 sieves, and weigh total residue on each sieve and also material passing thru the No. 60 sieve. With aid of Greenough-type microscope pick out and weigh the glass from fraction retained on No. 40 sieve. Estimate % glass in each portion passing thru No. 40 sieve.

NOTES. Check all particles of glass with polarizing microscope since particles of clear quartz may be mistaken for glass unless examined with polarized light. Since glass is isotropic in character it shows complete extinction of the transmitted light when examined between crossed nicols, while quartz, which is double refracting, exhibits polarization colors.

The estimation of % glass may be made by detg % glass particles from microscopic examination of min. of 200 random particles from each portion, using polarized light.

FRUIT AND FRUIT PRODUCTS

Apple Butter

36.045 Rot—Official

Make mold count as in **36.060.**

36.046 Insect and Rodent Filth—First Action

Weigh 100 g sample into 400 ml beaker, add enough hot H_2O to obtain uniform dispersion, and pour into 2 L trap flask, **36.001(o)**. Rinse beaker with hot H_2O and add rinse H_2O to flask. Add 25–35 ml castor oil and mix thoroly. Add enough hot H_2O to bring oil layer into neck of flask. Stir vigorously with vertical motion while adding H_2O. Let stand 30 min., trap off, filter, and examine paper microscopically.

Dried Apple Chops

36.047 Heavy Filth—First Action

Place 50 g sample in 1 L beaker or other suitable container. Add enough tap H_2O to cover apples and shake or stir 5 min. Empty contents of flask into No. 6 or No. 8 sieve, recovering H_2O in beaker. Wash out flask and add rinse H_2O to that in beaker. Rinse off pieces of apple with fine stream of tap H_2O delivered with as much force as possible. Catch this rinse H_2O in beaker also, transfer to 2 L separator, and let stand 15 min., with occasional gentle rotary shaking. Open stopcock, and draw off heavy particles and small quantity of liquid. Filter drained material and examine microscopically.

36.048 Insects and Light Filth—First Action

Place 50 g sample in beaker, cover with H_2O, and boil 15 min. Empty apples onto No. 6 or No. 8 sieve, recovering H_2O in beaker. Rinse beaker. Rinse apples with strong stream of hot H_2O. Place all H_2O in 2 L trap flask, **36.001(o)**, add 20 ml castor oil, mix well, and add enough hot tap H_2O to fill flask. Let stand 30 min. with occasional stirring. Trap off oil layer, add H_2O to flask, stir, and trap off again in 10 min. Filter trapped-off portion thru rapid paper. Examine microscopically.

Fresh, Canned, and Frozen Blackberries, Blueberries, Loganberries, Raspberries, and Cherries

36.049 Rot in Blackberries, Raspberries, and Other Drupelet Berries—First Action

(a) *Frozen with or without sugar.*—Pulp berries thru cyclone, **36.001(e)**, with screen openings 0.027″ diam. and mix thoroly. Mix 25 g pulp with 50 g 3% pectin soln, **36.002(aa)**. Make mold count as in **36.060.**

(b) *Frozen in sirup, canned in sirup or water.*—Drain berries 2 min. on No. 20 sieve. Pulp, dil., and make mold count as in (a).

36.050 Maggots in Blueberries and Cherries—Procedure

Weigh 567 g (20 oz) fresh fruit or use No. 2 can of processed fruit. Add 100 ml H_2O to fresh or frozen fruit and boil 5 min., with frequent stirring. (Omit this step with canned fruit.) Transfer ½″ layer of fruit to No. 6 sieve immersed in pan of H_2O. Shake loose maggots and debris thru sieve. Mash fruit carefully under H_2O in order to rub any remaining maggots thru sieve. Rinse and discard any pulp and seeds. Repeat above process with another portion of fruit.

After all fruit is screened, transfer mixt. to black-bottom pan. (With cherries, transfer first to No. 6 sieve resting in ca 1″ H_2O, shake sieve until maggots drop thru, and discard pulp on sieve.) Slowly decant H_2O and pulp from pan. Add more H_2O and repeat decantation. Pick out maggots found by careful examination of contents of pan. Transfer contents of this pan to white-bottom pan and pick out or count maggots found in this pan.

Strawberries (Frozen)

36.051 Mold—Official

Pulp thawed berries thru cyclone with screen openings 0.027″ diam. and mix thoroly. (Pour juice thru cyclone last.) If necessary, remove air bubbles with suction or by mixing ca 100 g pulp with 3–5 drops *capryl alcohol.* Again mix thoroly and make mold count as in **36.060.**

Fruit Paste

36.052 Light Filth—Procedure

Place 100 g paste in 2 L trap flask, **36.001(o)**, add ca 400 ml H_2O, and heat to boiling. Continue boiling ca 10 min., or until material is disintegrated; then cool to 20–25°. Add 30 ml kerosene and mix; then fill flask with H_2O. Let stand ca 30 min., stirring lower layer 5–6 times during this period. Trap off oily layer. Add 30 ml more kerosene, remix, and after stirring down upper layer several times, again trap off. Pour trapped-off material thru No. 20 sieve. Rinse sieve, filter material passing thru sieve, and examine. Rinse material retained on sieve into beaker, filter, and examine.

36.053 Insect Heads in Fig Paste and Fig Slices—Procedure

Place 100 g paste or fig slices in 2 L trap flask and add 500 ml warm (30–50°) H_2O. Boil, with

intermittent stirring, until paste is disintegrated and concd to ca 400 ml. Cool to 50–60°. Add 10 g NaOH, bring to boil, and continue boiling briskly ca 5 min. Cool to ca 50°. Add 20 ml HCl, and again cool to ca 50°. Add 30 ml castor oil, stir, and add H_2O until oil comes ca half way into neck of flask. Let stand 15 min. Agitate, and with plunger work down floating fruit pulp and seeds with downward motion to break interface. Repeat this operation twice. Add warm (ca 50°) H_2O to release those seeds at surface of oil. Let stand ca 30 min., stirring lower layers 5–6 times during this period. Trap off, filter, and examine microscopically.

Jam and Jelly

36.054 Insect and Rodent Filth—First Action

(a) *Jam.*—Empty contents of jar into dish and mix thoroly. Weigh 100 g into beaker, add 200 ml H_2O (ca 50°), transfer to 1 L trap flask, **36.001**(o), add 10 ml HCl, and boil ca 5 min. Cool to room temp., add 25 ml gasoline, and stir thoroly. Trap off, filter, and examine microscopically.

(b) *Jelly.*—Empty contents of jar into dish and mix thoroly. Weigh 100 g into beaker and add 300-400 ml hot H_2O; warm beaker, with stirring, until jelly dissolves, filter, and examine microscopically.

Occasionally so-called "jellies" contg small quantities of fruit tissue will not filter thru paper; in such cases proceed as in (a).

Cranberry Sauce

36.055 Mold —First Action

(a) *Strained sauce.*—Immerse unopened can of sauce in boiling H_2O bath 30–45 min. to facilitate breaking gel. Remove can from bath and open carefully to avoid loss of sauce thru sudden release of pressure. Transfer contents into suitable beaker (1 L beaker for No. 2 can). Stir sauce to break gel. (Slow-speed elec. mixer (350–450 rpm) may be used for this purpose.) Mix 50 g stirred sauce thoroly with 50 g 3% pectin soln. Make mold count of mixt. as in **36.060**.

(b) *Whole sauce (seeds and skins included).*—Pulp contents of container (if considerably >1 lb, such as No. 10 can, remove well-mixed aliquot of 1 lb) thru cyclone with screen openings 0.027″ diam. to remove skins and seeds, and prep. homogeneous pulp. Mix 50 g of this pulp with 50 g 3% pectin soln. Make mold count as in **36.060**.

SUGAR AND SUGAR PRODUCTS

Candy

36.056 Filth—First Action

(a) *In hard candy, gum drops, gum, starch, or pectin-base candies.*—Dissolve in boiling HCl (1+70), filter thru rapid paper on büchner, and examine microscopically.

(b) *In hard candy difficult to filter by* (a) *(e.g., licorice candy).*—Proceed as in (c) except to substitute HCl (1+70) for borax soln.

(c) *In chocolate candy with or without fruit or nuts, fruity candy, etc.*—Weigh 225 g sample into 2 L beaker, add 1 L 5–10% $Na_2B_4O_7.10H_2O$ soln, simmer 10–15 min., and pour thru 5–8″ No. 140 screen. While pouring, play forcible stream of hot H_2O onto material. Wash well with large stream of hot H_2O; wash twice alternately with alcohol and $CHCl_3$ in that order; finally rinse with alcohol and hot H_2O. Transfer material to filter paper if little residue remains or to 1–2 L trap flask, **36.001**(o), if quantity of residue is large.

In latter case, transfer bulk of material with spoon; then wash screen with forcible stream of hot H_2O so as to collect final residue at one edge of screen, and finally transfer residue to trap flask with stream of 60% alcohol from wash bottle. Add 600 or 900 ml 60% alcohol, depending on size of trap flask, boil 20 min., cool to <20°, and add 20 or 40 ml gasoline. Fill flask with 60% alcohol and trap off as usual. Add gasoline and trap off second time. Filter and examine. If large quantity of peanut testa or similar material floats into neck of flask, pour trapped-off material thru No. 8 or No. 10 sieve, rinse thoroly, filter liquid portion, and examine microscopically.

(d) *In chocolate candy coating.*—Heat 400 ml gasoline in 800 ml beaker to 40–50° and keep at this temp. Place portion of candy in wire basket (ca $3\frac{1}{4}$″ diam.×1″ high) made from No. 8 screen and with wire handles. Move basket up and down thru gasoline until chocolate coating dissolves. Rinse each candy center with fine stream of gasoline from wash bottle and save center. Repeat with balance of sample. Stir gasoline-chocolate suspension and pour thru No. 140 sieve. Transfer residue from sieve to filter paper and examine microscopically. Examine candy centers by appropriate method, (a), (b), or (c).

Chewing Gum

36.057 Filth—Procedure

Add 100 g gum to 300–600 ml H_2O or 150–600 ml HCl (1+17), bring to boil, and boil 10–12 min. To prevent excessive caramelization, do not boil longer than necessary to obtain fine dispersion of gum particles in the liquid. Treat dispersed gum by one of following procedures to dissolve or soften chicle:

(a) Let mixt. cool to ca 55°, add 150 ml acetone, and stir; then add 150 ml $CHCl_3$. Bring to boil and boil until mixt. is dispersed evenly. While hot, pour thru 10XX bolting cloth in Hirsch funnel, and examine microscopically.

(b) Cool to ca 80° (if CCl_4 is to be used) or ca 63° (if $CHCl_3$ is to be used); add ca 150 ml CCl_4

or $CHCl_3$ and simmer 5–10 min. or until chicle dissolves. While hot, filter thru 10XX bolting cloth in Hirsch funnel, and examine microscopically.

Caution: As these mixts tend to foam and boil over, take adequate precautions against fire or hazardous prolonged breathing of vapors from the org. solvents. Stir all mixts while adding liquids and while heating to boiling. When transferring to new container, take care to rinse old container with hot H_2O and appropriate org. solvent. Filtering funnel may be greased to prevent the cooling gum from sticking to it.

Sugars

36.058 Filth—First Action

Dissolve 100 g sample in ca 200 ml hot H_2O. Boil, and filter at once thru rapid paper in büchner. Examine microscopically.

Sirups, Molasses, and Honey

36.059 Filth—First Action

(a) Mix sample thoroly and dissolve 200 g in 200 ml hot H_2O acidified with 5 ml HNO_3. Filter at once thru 7 cm rapid paper in suction funnel. Wash with min. quantity of hot H_2O and examine microscopically.

(b) *Alternative procedure.*—Dissolve 200 g in 500 ml hot H_2O. Filter at once thru 10XX bolting cloth in suction funnel. Wash with min. quantity of hot H_2O and examine microscopically.

VEGETABLES AND VEGETABLE PRODUCTS

Tomato Products (Not Dehydrated)

36.060 Molds (*8*)—Official

In making mold counts of tomato products, use juice and catsup as it comes from container. In case of purée and paste add H_2O to make mixt. with total solid content that gives immersion refractometer reading at 20° of 45.0–48.7 or refractive index at 20° of 1.3447–1.3460.

Clean Howard cell, **36.001**(j)(1), so that Newton's rings are produced between slide and cover glass. Remove cover and with knife blade or scalpel place portion of well-mixed sample upon central disk; with same instrument, spread evenly over disk, and cover with glass so as to give uniform distribution. Use only enough sample to bring material to edge of disk. (It is of utmost importance that portion be taken from thoroly mixed sample and spread evenly over slide disk. Otherwise, when cover slip is put in place, insol. material, and consequently molds, may be more abundant at center of mount.) Discard any mount showing uneven distribution or absence of Newton's rings, or liquid that has been drawn across moat and between cover glass and shoulder.

Place slide under microscope and examine with such adjustment that each field of view covers 1.5 sq. mm. (This area, which is essential, may frequently be obtained by so adjusting draw-tube that diam. of field becomes 1.382 mm. When such adjustment is not possible, make accessory drop-in ocular diaphragm with aperture accurately cut to necessary size. Diam. of area of field of view can be detd by use of stage micrometer. When instrument is properly adjusted, quantity of liquid examined per field is 0.15 cu. mm.) Use magnification of 90–125×. In those instances where identifying characteristics of mold filaments are not clearly discernible in std field, use magnification of ca 200× (8 mm objective) to confirm identity of mold filaments previously observed in std field.

From each of 2 or more mounts examine at least 25 fields taken in such manner as to be representative of all sections of mount. Observe each field, noting presence or absence of mold filaments and recording results as positive when aggregate length of not >3 filaments present exceeds $\frac{1}{6}$ of diam. of field. Calc. proportion of positive fields from results of examination of all observed fields and report as % fields contg mold filaments.

36.061 Yeasts and Spores—Official

Fill graduated cylinder with H_2O to 20 ml mark and add sample until mixt. reaches 30 ml mark, to obtain diln of 1+2. Close graduate, or pour contents into erlenmeyer, and shake vigorously 15–20 sec. To assure thoro mixing, mixt. should fill not $>\frac{3}{4}$ of erlenmeyer. For tomato sauce or pastes, or for products running high in number of organisms, or of heavy consistency, use diln that permits ready counting.

Pour mixt. into beaker. Thoroly clean blood-counting cell, **36.001(a)**, to obtain good Newton's rings. Thoroly stir contents of beaker with scalpel or knife blade and after it stands 3–5 sec., remove small portion, place it upon central disk of blood-counting cell, and cover immediately with cover glass. Discard any mount showing uneven distribution, absence of Newton's rings, or liquid that has been drawn across moat and between cover glass and shoulder. Let slide stand not <10 min. before beginning to make count. Use magnification of 300–400×. Count number of yeasts and spores in either whole counting area (1.0 sq. mm), which represents vol. of 0.1 cu. mm, or portion thereof. If less than whole vol. is counted, choose representative areas for counting. Calc. to number of yeasts and spores/ml or g of original products.

Example: If 25 spores were observed in $\frac{1}{2}$ of 1.0 sq. mm area, and diln of 2 parts H_2O with 1 part product was made originally, then total number of organisms $= 25 \times 2 \times 10 \times 1000 \times 3 = 1{,}500{,}000$, where 25 = no. of organisms observed; 2 = factor to det. organisms/0.1 cu. mm; 10 = factor to det.

organisms/1.0 cu. mm; 1000 = factor to det. organisms/1.0 ml; and 3 = factor representing diln.

36.062 Rot in Canned Tomatoes— First Action

Drain contents of can 2 min. on No. 2 sieve. For containers of <3 lb net wt, use 8″ diam. sieve; for containers of 3 lb or more net wt, use 12″ sieve. Examine drained tomatoes and record number and size of any rotten portions present. Pass drained tomatoes thru laboratory cyclone, **36.001(e)**, with screen openings ca 0.027″ diam. Make mold counts on both drained juice and pulped tomatoes as in **36.060**.

36.063 Rot Fragments in Comminuted Tomato Products (9)— First Action

Weigh 10 g juice (5 g purée or catsup, or 2 g paste) and transfer with 100 ml H_2O to 400 ml beaker. Add ca 2 ml crystal violet soln, **36.002(m)** (10% crystal violet in alcohol may replace the aq. soln when latter fails), stir, and let stain 3 min. Add ca 200 ml H_2O, stir, and pour thru No. 60 sieve ca 7.5 cm diam., held in horizontal position. Pour material over entire surface of sieve, using glass rod held against lip of pouring beaker, with lower end of rod ca 2 cm from screen. If sample wt specified does not drain rapidly, reduce size of sample.

Rinse beaker with 200 ml H_2O and pour rinse H_2O over tomato debris on sieve, using glass rod as before. Tilt sieve to ca 30° angle and wash debris to lower part with ca 100 ml H_2O. Let debris drain, and transfer by means of spatula to bottom of graduated tube, ca 3×12 cm. Transfer remaining debris by washing down with H_2O from dropper and immediately taking up debris in wash H_2O before it has run thru screen. When completely transferred, make vol. H_2O and debris to 10 ml with H_2O. Add enough algin soln, **36.002(aa)**, to bring vol. to 20 ml. Mix stained suspension well, measure out 2 sep. 0.5 ml portions, and spread over 2 counting slides, **36.001(m)**. Examine each slide with microscope, **36.001(k)**(2), at 40–45× with transmitted light.

Rot fragments are tomato tissue to which mold filaments are attached. Some may appear as almost solid masses of mold. *See* Figs. 80 and 81. Count number of rot fragments on each of the 2 slides, add, and multiply by 2 (10 g sample), 4 (5 g sample), or 10 (2 g sample) to obtain number of rot fragments/g product.

36.064 Fly Eggs and Maggots— First Action

(a) *In comminuted products.*—Mix sample thoroly and transfer 100 g to 2 L separator. Add 20–30 ml gasoline and shake thoroly, releasing pressure as necessary. Fill separator with H_2O in such manner as to produce max. agitation. Place separator in ring stand and let settle; at 15 min. intervals during 1 hr, drain 15–20 ml from separator, and gently shake separator with rotary motion to facilitate settling out of fly eggs and maggots. If drained liquid contains seeds, pass it thru No. 10 sieve, and rinse seeds and sieve thoroly, recovering both liquid portion and rinse H_2O in beaker. Filter thru 10XX bolting cloth in Hirsch funnel. Examine for eggs and maggots at ca 10×. If fly eggs or maggots are found in this examination, continue sepg and draining as above addnl hr.

(b) *In canned tomatoes.*—Pulp entire contents of can in such way that min. number of eggs and maggots are crushed or broken. (This may be done by passing material thru No. 6 or No. 8 sieve and adding seeds and residue remaining on sieve to pulp.)

Place 500 g of the well-mixed pulped tomatoes in 6 L separator. Add 125–150 ml gasoline and ca 1 L H_2O and shake vigorously, releasing pressure as necessary. Fill separator with H_2O. Place separator in ring stand and let layers sep. At 15 min. intervals during 1 hr, drain 25–30 ml from bottom of separator, and gently shake separator with rotary motion to facilitate settling of fly eggs and maggots. Each portion may be examined at once or combined with subsequent portions. Pass drained portions thru No. 10 sieve and rinse seeds and sieve thoroly, recovering both liquid portion and rinse H_2O in beaker. Filter thru 10XX bolting cloth in Hirsch funnel. Examine cloth for eggs and maggots at ca 10×. If fly eggs or maggots are found in this examination, continue sepg and draining as above addnl hr.

36.065 Insect Fragments—First Action

Place 200 g of any tomato product except paste (where 100 g is used) in trap flask, **36.001(o)**, with 20 ml castor oil and mix well. Add enough warm tap H_2O (ca 50°) to fill flask. (At first, bubbles of air tend to bring up tomato tissues, but after several stirrings these begin to settle out, leaving H_2O layer near oil fairly clear.) Let stand with occasional gentle stirring 30 min.; then trap off into beaker. Wash out neck of flask with alcohol to remove adhering castor oil. Add to flask little more hot H_2O, stir, let stand 10 min., and then trap off again. (Occasionally it may be necessary to return trapped-off material to another trap flask and rewash to eliminate tomato tissue.) Filter trapped-off portion; wash beaker, sides of funnel, and paper thoroly with alcohol to dissolve oil and speed filtration. Examine paper at 20–30×.

FIG. 80.—ROT FRAGMENTS FROM TOMATO PURÉE. 40×

Tomato Soup, Canned Spaghetti, Pork and Beans, and Similar Products Containing Tomato Sauce

36.066 Mold—First Action

(a) *Tomato soup with or without starch.*—Place unopened can in hot H_2O and heat until contents are thoroly warmed; then open. Transfer 10 ml thoroly mixed soup to 50 ml centrifuge tube and add 3 ml NaOH soln (1+1). If starch is absent, omit the NaOH. Stir until starch dissolves and tissues clear. Add enough H_2O to fill tube, and centrifuge. (Time required to centrifuge sample varies greatly. With centrifuge arm length of 5¼" and speed of ca 1600 rpm, ca 20 min. is required for av. sample. In heavy soups, gelatinizing of much starch sometimes interferes with proper settling out of solids during centrifuging. If liquid remains cloudy, it may be necessary to discard sample and start again with only 5 ml soup and add 3 ml of the NaOH soln.) When supernatant is

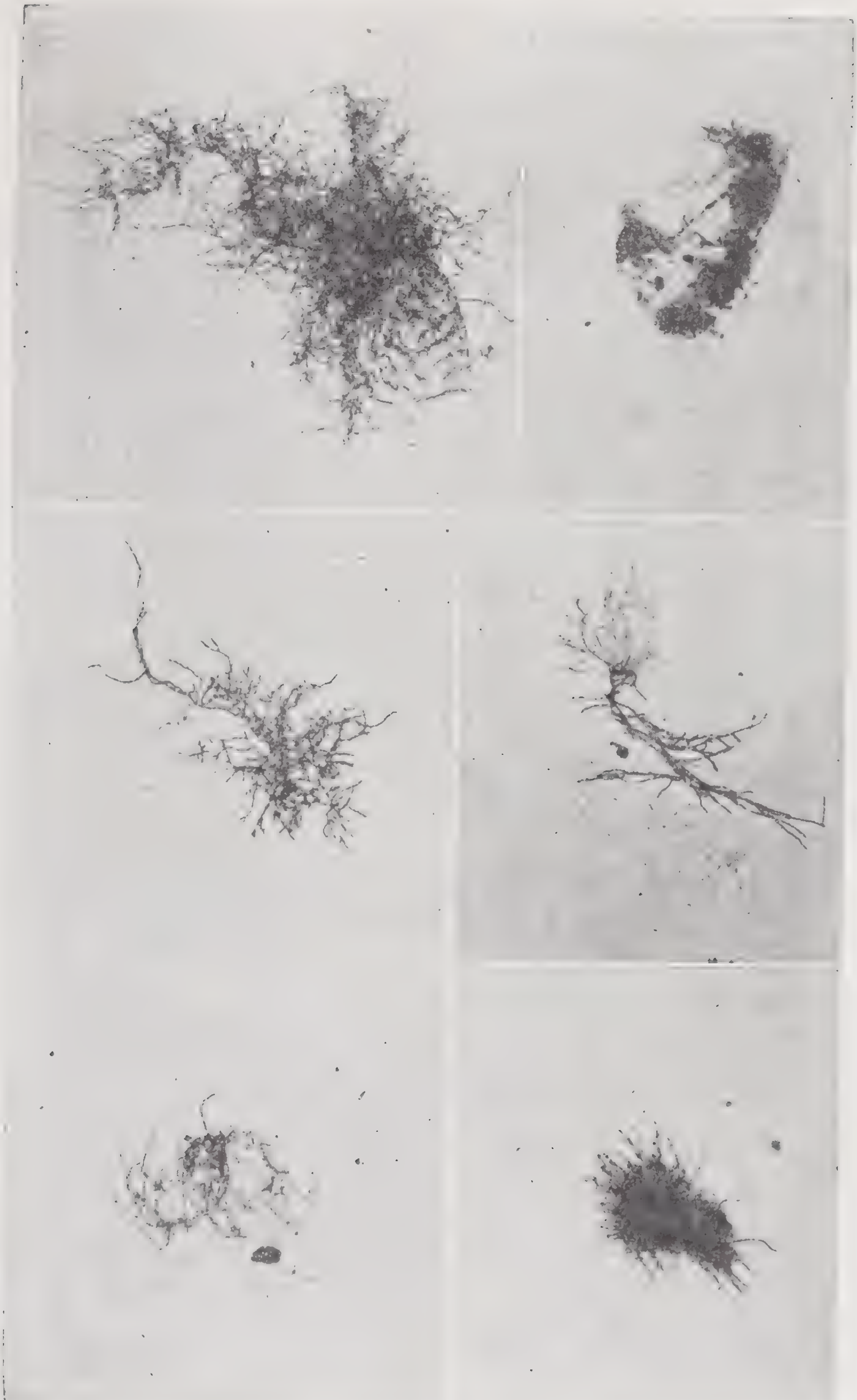

FIG. 81.—ROT FRAGMENTS FROM TOMATO PURÉE. 40×

clear, pour off; if not entirely clear, check supernatant for mold before discarding. Add enough H_2O to residue in tube to bring to original vol. of soup, mix, and count mold as in **36.060**.

(**b**) *Pork and beans, spaghetti with tomato sauce, spaghetti with meat balls or meat, ravioli, chili con carne, and tamales.*—Place unopened can in hot H_2O and heat until contents are thoroly warmed. Open can and transfer contents onto No. 6 sieve. Drain until major portion of liquid passes thru. (With some products, sauce runs thru at once, but in case of some beans and spaghetti 10 min. or more may be required.) Mix sauce thoroly, place 10 ml in centrifuge tube, and proceed as in (**a**). Use care in counting products contg meat so as not to confuse mold filaments and muscle fibers

that superficially resemble each other; muscle fibers are usually much thicker and striations are often visible.

(c) *Tomato sauce packing medium on fish.*—Place unopened can in hot H_2O (ca 90–95°) until contents are thoroly warmed. Open can and drain contents on No. 6 sieve until major portion of sauce and oil passes thru. Mix liquid and place up to 50 ml in 50 ml centrifuge tube and centrifuge as in (a). Record vol. of lower oil-free sauce layer and discard oil and part of aq. layer. Add H_2O to bring to noted vol., mix, and count mold as in **36.060**, removing bits of fish tissue from slide, if necessary, before counting.

Puréed Infant Food

36.067 Molds—First Action

Proceed as in **36.060.** Add ca 0.2 g NaOH to ca 6 g product before counting, and stir thoroly until NaOH is dissolved.

36.068 Insect and Rodent Filth—First Action

Utilize oil-in-H_2O extn principle as carried out in trap flask, **36.001(o)**. Analyze all foods in similar manner, except to vary temp. and type of oil used as indicated in table.

Transfer contents of 2 cans or jars of food to 1 L trap flask previously rinsed with H_2O. Thoroly mix in ca 20 ml of the oil. Fill with deaërated H_2O either at room temp. or at 50–70°. Let mixt. stand 30 min., stirring 4–6 times during this period to release filth from layer of food. Trap off and examine microscopically.

Use type of oil and temp. indicated in following table:

Food	*Oil*	*Temp.*
All fruits, Asparagus, Beets, Carrots, Green beans, Peas	Light mineral oil	Room
Spinach	Light mineral oil	50–70°
Squash	Castor oil	50–70°

36.069 Fly Eggs and Maggots—First Action

Transfer residue in the trap flask, **36.068,** to 2 L separator. Add ca 100 ml gasoline and shake vigorously. Let material settle ca 2 hr, stirring surface layer occasionally to permit any eggs and maggots to settle out. Withdraw ca 200 ml from bottom of separator and filter this material thru 10XX bolting cloth, using several cloths if there is large accumulation of sediment. Examine microscopically at 15–20×.

Peas and Beans

36.070 Weevils—First Action

Microscopic examination.—If the peas or beans are canned and of normal texture, pour on No. 8 sieve in pan filled with enough H_2O to stand 2–3 cm above mesh of sieve. Mash peas thru sieve with fingers. After as much as possible of material has been worked thru, remove sieve from pan and shake excess H_2O back into pan. Transfer material retained on sieve to No. 10 can. Pour material that passed thru No. 8 sieve onto No. 40 sieve, discarding that which passes thru. Let material on sieve drain few min., and shake lightly to remove free H_2O from solid material. (If peas are unusually hard, or have tough skins, pass contents of can thru meat or food chopper directly onto No. 40 sieve.) Discard any excess H_2O passing thru this sieve. Cook dried or frozen peas before maceration.

Add material retained on the No. 40 sieve to the No. 10 can. Add ca 130 ml gasoline to this material and mix thoroly with large spoon. Rinse any material remaining on sieve into can with H_2O. Stir material in can and pick out any insects that may rise to top of H_2O layer. Repeat stirring and searching several times until no more larvae are recovered.

Add enough H_2O to bring contents of can to within 1–2 cm of top. Pick out any larvae visible at surface. Stir again, let mixt. stand ca 5 min., and then skim off gasoline and upper part of H_2O layer with spoon and place in trap flask, **36.001(o)**, previously filled ca $\frac{1}{4}$ full of H_2O. Add 90–100 ml gasoline to material remaining in No. 10 can, and stir vigorously. After standing ca 5 addnl min., skim off gasoline and upper part of H_2O layer as before and add to material already in trap flask.

Fill flask with H_2O. Trap off as much as possible of gasoline and filter into Hirsch funnel or büchner. Lower stopper into flask, and, to rinse sides of trap flask, apply vac. ca 5 min. by fitting large rubber stopper and glass tube over mouth of flask. (As ordinary erlenmeyer collapses under vac. of 20″ of Hg, use either less vac. or heavy-wall flask.) Release vac., add H_2O, and trap off. Add trapped-off portion to that already on filter. Examine microscopically.

Potato Chips

36.071 Filth—First Action

Weigh 100 g sample into 1.5 L beaker. Crush chips into small pieces and cover with petr. ether. Let stand ca 5 min. and decant thru filter. Add petr. ether and decant again thru filter. Let petr. ether evap. from chips. Transfer to 2 L trap flask, add 500 ml 60% alcohol, and boil ca 30 min., replacing alcohol lost by evapn. Cool, add 35 ml gasoline, mix, let stand ca 5 min., and fill with

60% alcohol. Let stand, trap off twice, and filter as usual. Examine papers microscopically.

Canned Greens and Broccoli

36.072 Insects—First Action

Transfer contents of can to pan of suitable size and chop up leaves into pieces 1–2″ long. Weigh 100 g well-mixed sample into 1 L beaker. Add 500–600 ml H_2O and boil 5 min. Pour H_2O and sample into 2 L trap flask, **36.001(o)**. Add 35 ml gasoline and stir mixt. thoroly to insure contact between gasoline and all portions of leaves. Fill flask with deaërated H_2O, let stand 30 min., trap off gasoline layer, filter, and examine microscopically. Add 40 ml gasoline to flask and repeat extn.

To overcome difficulty caused by leaves occasionally rising to interface, place No. 8 sieve, 6–8″ diam., in suitable size evapg dish contg enough H_2O to cover screen ca ½″. Pour entrapped gasoline from trap flask onto sieve as it is held under the H_2O. Move sieve gently up and down to let insects pass thru into the H_2O. Remove screen and filter contents of dish. Repeat washing procedure to free any insects left on greens on screen, and filter washings. Examine papers microscopically.

36.073 Aphids—First Action

Det. drained wt of contents of canned greens as in **36.062,** reserving drained liquor. Chop drained leaves into pieces 1–2″ long and weigh 100 g well-mixed sample into 1 L beaker. Add H_2O to cover adequately, followed by 25 g neutral $Pb(OAc)_2.3H_2O$ crystals (or equiv. soln of $Pb(OAc)_2$) and 10 ml HOAc. Boil on hot plate 5–10 min., cool, and transfer to 2 L trap flask, **36.001(o)**. Add 35 ml gasoline, and mix thoroly to assure contact between gasoline and all portions of leaves. Fill flask with deaërated H_2O. Let settle few min. for most of vegetable matter to sink to bottom. To force any tissue that rises (probably held by entrapped globules of gasoline) to sink, pivot lower end of trap-rod on bottom of flask, and rotate upper part of rod around neck of flask to knock globules from vegetable tissue without at same time breaking interface and thus rewetting tissue with gasoline. Again let flask stand, trap off gasoline layer, and filter.

Re-ext. with 20 ml gasoline, trap off, and filter (usually possible on same paper). Det. total number of aphids or other light filth in entire liquor drained from can by subjecting it to gasoline flotation as usual. (Normally liquor does not present any difficulty and use of $Pb(OAc)_2$ is unnecessary.) Count aphids and thrips if heads are present, even tho they may be mutilated or crushed; up to one-half of any insect may be missing, but do not include in count cast skins and fragments of less than one-half. (Cast skins are recognizable by their pale, ghost-like appearance and complete absence of interior.) Count separately fragments of insects other than aphids and thrips, and other evidence of filth. Calc. on basis of wt drained material.

36.074 Heavy Filth—First Action

Recover heavy filth such as soil, maggots (especially those of spinach leaf miner), and rodent excreta, that sink to bottom of trap flask, as follows: Transfer contents of trap flask, **36.072** or **36.073,** by rinsing with H_2O into 4–6 quart pail. Add H_2O to pail until ca full. Stir, let stand short time, and decant ca half pail contents. Refill pail with H_2O and repeat operation until most of floating greens are removed. Wash into black shallow pan the heavy filth left in pail and examine visually for larvae, stones, and other debris, picking material out with forceps.

Sauerkraut

36.075 Filth—First Action

Use entire contents of containers of <2 lb. Use 24 oz well-mixed sample from larger containers. Wash thoroly, small portion at time (ca 4 oz), on 8″ No. 8 sieve nesting in 8″ No. 20 sieve with washings finally passing thru 8″ No.140 sieve. Wash material remaining on No. 20 sieve with washings passing thru No. 140 sieve. Transfer material on No. 20 sieve to paper and examine at ca 10× for whole insects or large body parts. Transfer material remaining on No. 140 sieve to paper and examine microscopically.

Mushrooms

36.076 Filth—Procedure

(a) *Canned.*—Place all mushrooms on No. 6 sieve in deep evapg dish. Add H_2O until sieve is ca half immersed. Thoroly break up mushroom pieces by rubbing over sieve. Agitate sieve while under H_2O, remove sieve, and filter liquid thru rapid paper. Examine paper or papers microscopically at 20–30×.

(b) *Dried.*—Thoroly mix sample and weigh 100 g portion. Transfer mushrooms to trap flask, **36.001(o)**, add H_2O, and let soak several hr, preferably overnight on steam bath, or boil 30 min. Cool to room temp., add 30 ml gasoline, and churn contents by hard, rapid pounding of mushrooms against bottom of flask, using vertical movement of rubber plunger. Trap off twice, filter, and examine microscopically.

Pour remaining liquid and mushrooms in flask onto No. 8 sieve in dishpan and add enough H_2O to partially immerse sieve. Rub mushrooms over sieve to release any insects, such as maggots, that may have remained in tunnels in mushrooms,

letting them drop thru sieve. Filter thru 10XX bolting cloth and examine microscopically.

36.077 Maggots—Procedure

For gross contamination, place 100 g sample in beaker, cover with hot H_2O, and boil 30 min. Pour onto No. 8 sieve in suitable pan, and add enough H_2O to cover. Release filth by rubbing mushrooms on sieve. Filter thru 10XX bolting cloth. Repeat extn process as many times as necessary to recover all heavy filth. Examine cloth microscopically.

Sweet Corn

36.078 Filth—First Action

(**a**) *Microscopic examination.*—Place 200 g well-mixed sample in 2 L trap flask, **36.001(o)**, add 20 ml castor oil, and mix well. Add enough hot tap H_2O (ca 50°) to fill flask. Let stand 30 min. with occasional gentle stirring; then trap off, into beaker, oil and H_2O layer and any corn debris that rises into neck of trap flask. To dissolve adhering oil, wash out neck of flask with hot alcohol. Add ca 10 ml more hot H_2O to flask, stir, let stand 10 min., and trap off again into same beaker.

Add ca 25–30 ml gasoline to the trapped-off portion and stir well to dissolve castor oil. Transfer contents of beaker into No. 6 or No. 8 sieve held in 400 ml beaker. Thoroly wash corn debris on sieve with hot alcohol, and filter material that passes thru sieve, washing beaker, sides of funnel, and paper thoroly with hot alcohol. Examine paper microscopically.

(**b**) *Macroscopic examination.*—Empty residue of corn remaining on bottom of flask when trapping is completed, (**a**), onto 5″ or 8″ No. 20 sieve. Place on sieve, portionwise, if necessary, remainder of corn from can, and wash under tap to remove starch and fine particles. Place residue on sieve in pan and examine under H_2O for worm-eaten or rotten kernels and whole worms, heads, or large fragments.

SPICES AND OTHER CONDIMENTS

Ground Allspice, Anise, Caraway, Cardamom, Celery Seed, Cloves, Coriander, Cumin, Curry Powder, Dill Seed, Fennel, Fenugreek, Ginger, Mace, Marjoram, Mustard, Nutmeg, Oregano, Poppy Seed, Rosemary, Sage, Savory, Thyme, Tea, Leafy Crude Drugs, and Condiments

36.079 Gross Contamination—First Action

Sift 200–400 g ground spice thru No. 20 sieve. Transfer any insects or other filth retained on sieve to suitable dish and examine with Greenough microscope.

36.080 Light and Heavy Filth—First Action

(**a**) *Heavy filth and sand.*—Weigh 10 g sample into 250 ml beaker. Add 150 ml petr. ether and boil gently 15 min. on elec. hot plate. Add petr. ether occasionally to keep vol. constant. Decant petr. ether onto smooth 7 cm paper in büchner. Add 150 ml $CHCl_3$ to beaker and let stand 30 min. with occasional stirring. Decant spice and $CHCl_3$ onto funnel, leaving heavy residue of sand and soil, if any, in beaker. If appreciable spice tissue remains on bottom of beaker, add successive portions of $CHCl_3$ mixed with CCl_4 to give increasingly higher sp. gr. until practically all spice tissue is floated off. Transfer residue from beaker to ashless paper and examine microscopically. If there is appreciable quantity of residue, place paper in weighed crucible, ignite, and weigh sand and soil.

(**b**) *Light filth.*—Dry material in büchner thoroly and transfer, including fine material that must be scraped from paper, to 1 L trap flask, **36.001(o)**. Add ca 150 ml H_2O, heat to boiling, and simmer 15 min., with stirring; wash down inside of flask with H_2O; and cool to <20°. Add 25 ml gasoline, mix thoroly, and let stand 5 min.; then fill flask with H_2O and let stand 30 min. Stir every 5 min., trap off, and filter. Add to flask ca 15 ml gasoline and mix thoroly; trap off and filter second time after 15 min. If second extn yields appreciable quantity of filth, decant most of liquid from flask, add 15 ml gasoline, and make third extn. Examine papers microscopically.

Ground Cinnamon

36.081 Light and Heavy Filth—First Action

(**a**) *Heavy filth and sand.*—Weigh 2 g sample into 50 ml centrifuge tube and add ca 45 ml CCl_4. Centrifuge 5 min. at 800 rpm in International size I, type SB, centrifuge, using No. 240 head with arm length of 5.25″, or equiv. Stir layer at top of liquid and repeat centrifuging. Decant ca $\frac{2}{3}$ of liquid and floating layer, and add fresh CCl_4 up to 45 ml. Mix thoroly and again centrifuge. Decant as much of liquid and floating layer as possible without disturbing residue in centrifuge tube. Wash residue onto 11 cm ashless paper with CCl_4. Examine under low-power microscope for filth. If there is appreciable residue, place paper in weighed crucible, ignite, and weigh sand and soil.

(**b**) *Light filth.*—Weigh 50 g sample into 600 ml beaker. Add 300–400 ml H_2O, stir until smooth, add filtered aq. ext. from 5 g pancreatin, **36.002(t)**, and mix. Adjust to pH 8 with Na_3PO_4 soln. Readjust pH after ca 15 min. and again after ca 45 min. Add 5 drops HCHO and digest overnight

at 37–40°. Cool, transfer digested material to 2 L trap flask, and add H_2O to 800 ml. Trap off twice with 25 and 15 ml gasoline, resp., as usual. Combine trappings in beaker, transfer to trap flask, and fill with H_2O. Stir, and after 30 min., trap off into beaker and filter. Examine microscopically.

Ground Turmeric

36.082 Light Filth—First Action

Weigh 25 g sample into 400 ml beaker. Add 300 ml $CHCl_3$-CCl_4 mixt. (1+1), stir thoroly, and let stand 15 min. with occasional stirring. Transfer mixt. onto 15 cm paper in büchner and rinse with solvent. Dry overnight or in oven 1 hr at 80°. Transfer dry residue to 600 ml beaker and proceed as in **36.081**(**b**).

Ground Onion and Garlic Powder

36.083 Light and Heavy Filth—First Action

(**a**) *Heavy filth and sand.*—Weigh 50 g sample into 250 ml hook-lip beaker. Add 200 ml CCl_4, stir thoroly, and let stand 30 min. with occasional stirring. Decant plant tissue onto 15 cm paper in büchner, add 100 ml CCl_4, and repeat decantation until practically no plant tissue remains with sand and soil on bottom of beaker. Transfer residue in beaker to ashless paper with stream of CCl_4 from wash bottle and examine for filth. If there is appreciable residue, place paper in weighed crucible, ignite, and weigh sand and soil.

(**b**) *Light filth.*—Dry residue of plant tissue from büchner, (**a**), overnight or in oven 1 hr at 80°, and transfer to 2 L trap flask. Add 250 ml Tween 80–60% alcohol soln, **36.002**(**bb**), mix well, and let stand 15–30 min. Add 60% alcohol to 800 ml and trap off twice in 60% alcohol with 75 and 35 ml gasoline, resp., as usual. Let stand 1–1.5 hr for each extn and avoid stirring except for few circular upward strokes immediately after filling flask with 60% alcohol. Filter, and examine microscopically.

Ground Black and White Pepper

36.084 Light and Heavy Filth—First Action

(**a**) *Heavy filth and sand.*—Weigh 50 g sample into 600 ml beaker. Add 400 ml CCl_4 and let beaker stand at least 1 hr with occasional stirring. Decant pepper and solvent onto 15 cm paper in büchner, leaving heavy residue of sand and soil in beaker. Repeat decantation with CCl_4 if necessary to secure practically complete sepn of spice materials from any heavy residue. Transfer residue from beaker to ashless paper and examine for filth. If there is appreciable residue, place paper in weighed crucible, ignite, and weigh sand and soil.

(**b**) *Light filth.*—Wash spice material in büchner (**a**), with $CHCl_3$ and dry overnight or in oven at 80°. Transfer dry residue to 600 ml beaker and proceed as in **36.081**(**b**).

Ground Capsicums (Red and Cayenne Pepper, Chili Powder, Paprika, etc.)

36.085 Light and Heavy Filth—First Action

(**a**) *Heavy filth and sand.*—Isolate gross filth such as large larvae, adult insects, clumps of webbing, and insect and rodent excreta pellets by sifting pepper thru No. 10 sieve.

Weigh 50 g sifted sample into 600 ml beaker and add 400 ml petr. ether. Boil gently 30 min., adding petr. ether occasionally to keep vol. constant. Decant petr. ether onto smooth 15 cm paper in büchner. Add 400 ml CCl_4 and let stand 30 min. with occasional stirring. Decant pepper and solvent onto same 15 cm paper in büchner, leaving heavy residue of sand and soil in beaker. Repeat decantation with CCl_4 if necessary to secure practically complete sepn of spice materials from heavy residue. Transfer residue from beaker to ashless paper and examine for filth. If there is appreciable residue, place paper in weighed crucible, ignite, and det. sand and soil.

(**b**) *Light filth.*—Wash spice material in büchner, (**a**), with $CHCl_3$ and dry overnight or in oven at 80°. Transfer dry residue to 600 ml beaker and add 300–400 ml H_2O, stirring until smooth. Add filtered aq. ext. from 5 g pancreatin, **36.002**(t), and mix. Adjust to pH 8 with Na_3PO_4 after ca 15 min., and again after ca 45 min. Add 5 drops HCHO and digest overnight at 37–40°. Transfer digested material to 2 L trap flask, cautiously boil ca 10 min. until foaming partially subsides, and cool to 20°. Add H_2O to ca 800 ml and trap off twice with 25 and 15 ml gasoline, resp., as usual. Combine trappings in beaker, transfer to trap flask, and fill with H_2O. Stir, and after 30 min., trap off into beaker and filter. Examine microscopically.

36.086 Rot (Based on Mold Count)—First Action

Weigh 10 g thoroly mixed sample of ground capsicum and transfer to high speed blender. Add 200 ml 1% NaOH soln in 3 or 4 successive portions, stirring after each addn, washing down with final portion any material that may stick to walls of blender. Agitate mixt. in blender 1 min. With rubber policeman rub down into mixt. any material sticking to walls and repeat blending 2 min. longer. Add 2 or 3 drops *capryl alcohol* to break foam. Mix 100 g of this mixt. with 50 g 3% pectin soln, **36.002**(**aa**), and count with Howard mold-counting slide, **36.001**(**j**)(**1**), as in **36.060**.

Occasionally blended mixt. contains particles of seed tissue that make it difficult to obtain Newton's rings in prepg slide for mold counting. Clamp devised for holding cover slip in place to obviate this difficulty consists of metal plate with circular opening, 2.5 cm diam., in center of plate; 2 clips attached to edge of plate hold cover slip in position when slide is placed on plate.

Whole Spices

36.087 Filth by Flotation—First Action

Crack whole spice into small pieces to facilitate extn and proceed as in **36.080**, using 100 g sample with appropriate changes in app. size and reagent vols.

Whole Marjoram, Savory, and Thyme

36.088 Filth—First Action

Weigh 25 g sample into 400 ml beaker and proceed as in **36.080**(a) and (**b**), except use more reagent, and where necessary, 2 L trap flask, and add 400 ml hot $H_2O + 20$ ml HCl; also use 35 ml gasoline instead of 25 ml.

Whole, Cracked, or Pieces of Allspice, Anise, Bay Leaves, Caraway, Celery Seed, Cinnamon, Cloves, Coriander, Cumin, Dill Seed, Fennel Seed, Fenugreek, Ginger, Mace, Mixed Pickling Spice, Mustard, Nutmeg, Oregano, Black Pepper, White Pepper, Poppy Seed, Rosemary, Sage, and Turmeric

36.089 Filth—First Action

Weigh 25 g sample and proceed as in **36.080**(**a**) and (**b**), using larger beaker and more reagent if necessary.

Unground Fermented Crushed Peppers

36.090 Light and Heavy Filth— First Action

(**a**) *Light filth.*—Mix in beaker 100 g of the peppers with 100 ml gasoline. Thin mixt. with little H_2O if necessary for mixing. Pour mixt. into cylindrical, white enamel pan ca 8″ high × 10″ diam. that has been almost filled with H_2O, and stir gently. Most of pepper particles sink or remain suspended in the H_2O; light filth and some debris come to surface with the gasoline. Decant top layer of gasoline and part of H_2O layer (ca 1.5 L in all) into 2 L trap flask, **36.001**(**o**), thru glass funnel. Rinse funnel and fill trap flask with H_2O. Stir, and let settle 30 min. Trap off, filter, and examine microscopically.

(**b**) *Heavy filth.*—Gently stir material in pan (**a**), and let settle ca 30 sec. Decant pepper skin fragments. Add more H_2O and repeat operation until quantity of seeds and pepper fragments does not seriously interfere with examination of paper for heavy filth. Do not try to get paper entirely free from pepper skin and seeds, because filth will also be decanted. Transfer heavy residue to 7 cm paper and examine microscopically.

Pepper Sauce

36.091 Light and Heavy Filth— First Action

(**a**) *Light filth.*—Mix 100 g sauce with 100 ml H_2O and 35 ml gasoline in 2 L trap flask, **36.001**(**o**). Fill flask with H_2O, stir, and let settle 30 min. Trap off, filter, and examine microscopically.

(**b**) *Heavy filth.*—Transfer remainder of material from (**a**) in trap flask to white enamel pan. Treat by sedimentation method as in **36.090**(**b**).

Prepared Mustard

36.092 Filth—First Action

Weigh 100 g sample into 1 L beaker. Add 500 ml filtered pancreatin soln, **36.002**(**t**). Add enough $NaHCO_3$ to bring mixt. to pH 8 and digest 2 hr at ca 40°. Wash mixt. thru No. 100 sieve with hot H_2O until drainings are clear. Wash residue on sieve with alcohol followed by $CHCl_3$ and hot H_2O. Transfer to 1 L trap flask, **36.001**(**o**), by washing sieve with 60% alcohol. Add 25 ml gasoline, mix thoroly, and let stand 5 min. Fill flask with H_2O and let stand 30 min. Stir every 5 min. Trap off, filter and examine microscopically.

Condimental Seeds

36.093 Rodent and Insect Excreta— First Action

Prep. liquid with sp. gr. of 1.16–1.19 by mixing $CHCl_3$ or CCl_4 with alcohol or petr. ether. Mix 200 g of the seed with 500–700 ml of the liquid in 1 quart drug percolator. Let mixt. stand 30 min., stirring at ca 5 min. intervals. Trap sediment in lower end of percolator with cork plug and remove lower cork so as to deliver all sediment into beaker. Lift upper cork slightly and rinse tube and cork by letting small quantity of liquid pass. After stirring top layer, make 2 more sepns at 5 min. intervals. Transfer contents of beaker to filter paper, drain off liquid, and examine. Sep. rodent excreta and insect excreta, air dry, and weigh each separately to nearest mg.

Whole Tamarind Pulp

36.094 Light Filth—First Action

Mix sample thoroly and weigh 500 g into 1.5 L beaker. Add hot H_2O to within 1″ of top of beaker and simmer 15–20 min., stirring occasionally to break up mass of material. Pour contents of beaker thru No. 2 sieve, catching filtrate in convenient receptacle.

Break up material on sieve and wash thoroly

with hot H_2O to remove all small adhering particles (filth, etc.). Discard material retained on No. 2 sieve, pour material passing thru sieve onto No. 100 sieve, and wash thoroly with hot H_2O.

Transfer material retained on No. 100 sieve to 2 L trap flask, **36.001(o)**, with cold H_2O. Mix in 35 ml gasoline and let stand 5 min. Fill flask with H_2O and let settle 30 min., stirring every 5 min. (Pulp rising in neck of flask may be worked down by stirring gently with rubber stopper.) Trap off, and filter thru 10XX bolting cloth. Add to flask ca 20 ml gasoline and mix thoroly; trap off and filter second time after 15 min. If second extn yields appreciable quantity of filth, decant most of liquid from flask, add 15 ml gasoline, and make third extn. Examine papers microscopically.

Whole Pickles

36.095 Filth—First Action

Pour entire contents of jar onto No. 8 sieve nested in No. 100 sieve. Wash jar thoroly to remove any filth adhering to sides, and pour washings thru sieves. Wash pickles thoroly with stream of hot H_2O, turning from time to time. Transfer material on No. 100 sieve directly to ruled paper and examine microscopically. If only small quantity of debris is being washed from pickles it may be washed directly onto filter paper. State which method was used.

Chopped Pickles and Relish

36.096 Filth—First Action

Add 200 ml H_2O to 100 g pickles in trap flask or beaker, boil 15 min., and cool. If boiling is done in beaker, transfer to trap flask, **36.001(o)**. Add 25 ml kerosene, mix, and trap off. Filter, and examine microscopically.

Dressings for Food

36.097 Filth—First Action

Weigh 200 g sample into 800 ml beaker, stir in 50 ml H_3PO_4, and mix thoroly. Thin with ca 600 ml H_2O, and again mix thoroly. If possible, filter thru paper with suction, and examine paper microscopically. Filter salad dressing and materials not filterable thru paper thru No. 140 sieve, transfer filth to paper, and examine microscopically.

MISCELLANEOUS

Urine Stains on Foods and Containers (*10*)—First Action

36.098 *Preliminary Examination with Ultraviolet Light*

Examine suspected stains in dark room under ultraviolet light. (Dried urine on textiles usually fluoresces with blue-white color, but color varies somewhat, depending upon natural color of textile and type of lamp and filter used.) Run check patches with known types of urine. For microchemical analysis, outline stained area with pencil under the ultraviolet light. When odor of urine is detected, report this finding.

36.099 *Urease Test for Urea*

Cut out portion of stained area and transfer 1 or 2 threads to 5 ml crucible or beaker. Save balance of cloth for **36.102.** Leach 10 min. in just enough warm H_2O to cover material. Remove threads and squeeze out as much fluid as possible with clean, flat-tip forceps.

Transfer 2 or 3 drops to microculture slide with deep cylindrical depression. Add small drop *urease mixt.* (suspension of $\frac{1}{4}$ of 25 mg urease tablet in 0.5–0.7 ml H_2O). Place small drop *10% H_2PtCl_6 soln* on cover slip and invert over the depression, with hanging drop at center of depression opening. (Cover slip may be sealed on with petrolatum if only minute quantities of urea are suspected.)

With evolution of NH_3, brilliant, highly refractive, octahedral crystals of $(NH_4)_2PtCl_6$ are formed in hanging drop. Time required for crystals to form varies from few sec. to 30 min., or even longer in some instances, according to conditions. Crystals may be visible to naked eye and are readily detected under microscope at 100×. Certain org. compounds that are volatile and H_2O-sol. may yield crystals in the hanging drop, and if reagent soln is too concd, H_2PtCl_6 may crystallize. However, crystal habits of these substances are different from those of $(NH_4)_2PtCl_6$. (Stained patches of the food material can be tested by method similar to above.)

Urease-Bromothymol Blue Test Paper Test for Urea (11)—First Action

(Applicable to cloth or sack fibers, whole or ground cereal grains, whole or chopped nuts, spices, neutral solutions, etc.)

36.100 REAGENTS

(**a**) *Urease soln.*—Wet 0.2 g urease powder with small amount of H_2O, stir into paste, and dil. to 10 ml with H_2O.

(**b**) *Bromothymol blue soln.*—Rub 0.15 g indicator powder in mortar with 2.4 ml 0.1N NaOH soln. After indicator dissolves, wash mortar and pestle with H_2O, and dil. to 50 ml with H_2O. Soln should be green; pH ca 7.0.

(**c**) *Test paper A.*—Mix 10 ml indicator soln, (**b**), with 10 ml urease soln (**a**). Pour mixt. into watch glass. Using clean tweezers, dip pieces of heavy filter paper (Whatman No. 5, S&S No. 598, or 589 green ribbon have been found satisfactory) in soln. (To avoid uneven distribution of indicator and enzyme, wet entire paper at one time by laying it on surface of soln.) Hang paper

to dry in place free from NH_3 fumes, strong air currents, or heat. Paper should be orange when dry. Store dry paper in well-stoppered, dark glass bottle in cool place.

(**d**) *Test paper B*.—Dil. indicator soln, (**b**), with equal portion of H_2O. Dip pieces of filter paper (same kind as used for test paper A) in indicator soln and hang to dry as in (**c**).

36.101 QUALITATIVE TEST

Test neutral solns for urea by placing drop on dry test paper A. Appearance of blue or green color after few min. incubation at room temp. indicates urea.

For detection of urea in very small, dry particles, dip pieces of both test papers, A and B, of appropriate size into H_2O, using clean tweezers. Wet each entire paper at one time by laying it on surface of H_2O (indicator flows unevenly if paper is wet with drops). Shake papers to remove excess H_2O and place on clean, flat piece of glass. (If paper on glass has shiny appearance, too much H_2O has been added. Let dry slightly before using.) Place sample on papers, cover with another clean, flat piece of glass, and press down gently.

Immediate development of blue color on both papers A and B indicates alk. particles. If alk. particles are extremely small, color development is delayed 10–30 sec., but develops on both papers. Blue spots which develop on test paper A alone indicate urea. Reaction usually requires 30–60 sec. to give detectable color, time varying inversely with urea concn. Spots continue to develop and enlarge for 10–20 min. and then fade gradually.

Larger particles may be tested similarly, altho it may not be practical to cover them with glass plate. Papers must be protected from NH_3 fumes and from drafts that would remove liberated NH_3 from the urea.

36.102 *Xanthydrol Test for Urea*

Place portion of stained cloth, ca $\frac{1}{8}''$ square (stain located by fluorescence) on microscope slide. Add drop of HOAc (2+1) and stir. (Or instead of cutting out a patch of cloth, rinse stained material with H_2O or other suitable solvent such as HOAc, acetone, or hot alcohol, evap. soln to dryness, dissolve residue in little HOAc (2+1), and place drop on slide.)

Transfer droplet with stirring rod to another place on slide and dil. with drop of HOAc (2+1). To both drops add very small quantity of *xanthydrol* and stir into soln. If urea is present, crystals of dixanthyl-urea form very shortly. Examine with magnification of ca 100–120× (higher power may be used for closer examination if crystals formed are quite small). Use of polarizing microscope is desirable but not essential.

Crystals may be either or both of 2 kinds, depending on concn of urea present: (*a*) most prevalent are clusters of narrow feather-blades of low birefringence which form thruout soln at ca 1:200 to 1:25,000 concn (under low power they may appear to be needles or threads); (*b*) straight needles, often in sheaves or clusters, of much greater birefringence, forming chiefly at or near edge as drop evaps, at concns from 1:50 to 1:1,000. Both kinds have negative elongation (observed with polarizing microscope, using red plate). Crystals should be noted before drop dries, but remain when it dries. Response is given by fresh urine solids content of ca 2 mg to 4 mmg in drop. Test material from portion of sample other than fluorescent spot as blank.

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37. Microbiological Methods

EXAMINATION OF EGGS AND EGG PRODUCTS (*1*)

("Manual of Methods for Pure Culture Study of Bacteria" of Society of American Bacteriologists should be used as guide for further study of microorganisms obtained in culturing procedures described.)

Sampling—Official

37.001 EQUIPMENT

(**a**) *Liquid eggs.*—Sampling tube or dipper, sterile sample containers with tight closures (pint Mason jars or friction top cans are most practical), alcohol, alcohol lamp or other burner, absorbent cotton, clean cloth or towel, and H_2O pail.

(**b**) *Frozen eggs.*—Elec. (high speed) or hand drill with 1×16" auger, hammer and steel strip (12×2×0.25"), or other tool for opening cans; tablespoon, hatchet or chisel, precooled sterile containers, etc., as in (**a**).

(**c**) *Dried eggs.*—Grain trier long enough to reach to bottom of containers to be sampled. Clean sample containers with tight closures (pint Mason jars or paperboard cartons), clean cloth or towel, and tablespoon.

37.002 METHODS

Take samples from representative number of containers in lot, **16.001.** Sterilize sampling tube or dipper, auger, spoon, and hatchet by wiping with alcohol-soaked cotton and flaming over alcohol lamp or other burner. Between samplings, wash instruments thoroly, dry, and resterilize. (Grain trier and spoon used in sampling dried eggs need not be sterilized but should be wiped with clean cloth after each sampling.) Open and sample all containers under as nearly aseptic conditions as possible.

(**a**) *Liquid eggs.*—Mix contents of container thoroly with sterile sample tube or dipper, and transfer ca ¾ pint to sterile sample container. Keep samples at <5° but avoid freezing. Observe and record odor of each container sampled as normal, putrid, sour, or musty.

(**b**) *Frozen eggs.*—Remove top layer of egg with sterilized hatchet or chisel. Drill 3 cores from top to bottom of container: first core in center, second core midway between center and periphery, and third core near edge of container. Transfer drillings from container to sample container with sterile spoon. Examine product organoleptically by smelling at opening of fourth drill-hole made after removal of bacteriological sample. (Heat produced by elec. drill intensifies odor of egg material, thus facilitating organoleptic examination.) Record odors as normal, putrid, sour, or musty. Refrigerate samples with Dry Ice (solid CO_2) or other suitable refrigerant if analysis is to be delayed or sampling point is at some distance from laboratory.

(**c**) *Dried eggs.*—For small packages, take entire parcel or parcels for sample. For boxes and barrels, remove top layer with spoon or other clean instrument, and with clean trier remove at least 3 cores as in (**b**). (Samples should consist of ca ¾ pint.) Transfer core aseptically to sample container with clean spoon or other suitable instrument. Store samples under refrigeration or in cool place.

Culture Media—Official

37.003 STANDARD METHODS MEDIA

(**a**) *Tryptone glucose yeast agar.*—Bacto dehydrated, or prep. from 5 g tryptone, 1 g dextrose, 2.5 g yeast ext., 15 g agar, and 1 L H_2O; adjust to pH 7.0.

(**b**) *Milk protein hydrolysate glucose agar.*—BBL dehydrated, or prep. from 9.0 g milk protein hydrolysate, 1 g dextrose, 15 g agar, and 1 L H_2O; adjust to pH 7.0.

(**c**) Prep. following media as in "Standard Methods for Examination of Water and Sewage," 10th ed., 1955, American Public Health Association: Levine eosin methylene blue agar, Endo agar, tryptophane broth, lactose broth, Me red-Voges Proskauer peptone medium, and Koser Na citrate medium.

37.004 OTHER MEDIA

(**a**) *Malt agar.*—Malt ext. (Difco), 3% or 30.0 g; agar, 1.5% or 15.0 g; and H_2O, 1 L. Boil to dissolve medium. Autoclave 15 min. at 121°. Just before use, melt malt agar and acidify with 85% lactic acid. Do not reheat medium after addn of acid.

(**b**) *Buffered distilled water.*—To prep. stock soln, dissolve 34 g KH_2PO_4 in 500 ml H_2O, adjust to pH 7.2 with 1*N* NaOH (ca 175 ml), and dil. to 1 L with H_2O. To prep. buffered distd H_2O for dilns, dil. 1.25 ml stock soln to 1 L with boiled and cooled H_2O. Autoclave 15 min. at 121°.

(**c**) *Physiological salt soln.*—NaCl, 0.85%; or

8.5 g and 1 L H_2O. Autoclave mixt. 15 min. at 121°.

(d) *Veal infusion agar.*—Ground lean veal, 500.0 g, and H_2O, 1 L. Infuse overnight in refrigerator and strain thru cheesecloth without pressure. Dil. to original vol. with H_2O and skim off any fat. Steam in Arnold sterilizer 30 min. and filter thru paper. Add peptone (Difco), 1.0% or 10.0 g; NaCl, 0.5% or 5.0 g; and agar, 1.5% or 15.0 g.

Steam in Arnold sterilizer to dissolve ingredients. Adjust to pH 7.6 and steam in Arnold sterilizer 15 min. Filter thru büchner with paper pulp mat, with suction. (Use egg albumen for clarification when necessary. Add fresh white of 1 egg previously beaten with 50 ml of the medium or its equiv. in desiccated egg white (1.5 g) to each L of medium before adjusting pH and after cooling to 50°. Shake thoroly to insure soln of egg white. Let stand 20 min. Heat in Arnold sterilizer 15 min. to coagulate egg white. Shake vigorously and reheat. Filter, adjust to pH 7.6, steam in Arnold sterilizer 15 min., and filter.)

Place 10 ml portions in test tubes or 80 ml quantities into bottles. Autoclave 20 min. at 121°; final pH 7.4.

For hemolytic tests, cool melted agar to 45° and add 5% defibrinated horse, sheep, or rabbit blood prior to pouring plates (0.5 ml blood/10 ml medium).

Operating Technic (*2*)

37.005 PREPARATION OF SAMPLE—OFFICIAL

(a) *Liquid eggs.*—Thoroly mix sample with sterile spoon or sterile elec. stirrer and prep. 1–10 diln by aseptically weighing 11 g egg material into sterile wide-mouth g-s. or screw-cap bottle, add 99 g sterile buffered distd H_2O, **37.004**(b), or sterile physiological saline, **37.004**(c), and 1 tablespoonful sterile glass shot. Agitate 1–10 diln thoroly to insure complete soln or distribution of egg material in diluent by shaking each container rapidly 25 times, each shake being up-and-down movement of ca 1 foot, time interval not exceeding 7 sec. Let bubbles escape. Transfer representative portion from 1–10 diln for higher serial dilns as needed. Proceed as in **37.006–37.010**(a). Pour all plates and inoculate other media within 15 min. after prepn of first diln to prevent growth or death of microorganisms.

(b) *Frozen eggs.*—Thaw frozen egg material as rapidly as possible to prevent increase in number of microorganisms present and at temp. low enough to prevent destruction of the microorganisms (not >45° for not >15 min.). (Frequent rotary shaking of sample container aids in thawing frozen material. Thawing temp. may be maintained by use of H_2O bath or bacteriological incubator.) Proceed as in (a).

(c) *Dried eggs.*—Thoroly mix sample with sterile spoon or spatula. Prep. 1–10 diln as in (a). If material is relatively insol. (stored samples), use $0.1N$ LiOH as diluent. Prep. serial dilns as in (a) and proceed as in **37.006–37.010**(b).

37.006 PLATE COUNTS—OFFICIAL

Inoculate one set of Petri plates with 1 ml portion of each suitable diln. Pour plates with tryptone glucose yeast agar or milk protein hydrolysate glucose agar previously cooled to 42–45°. Incubate inoculated plates 3 days at 32°. Count plates with aid of Quebec colony counter, if available. Express final results as number of viable microorganisms/g egg material.

37.007 INCIDENCE OF COLIFORM GROUP—OFFICIAL

(a) Inoculate 1.0 ml portions from suitable dilns of egg material into fermentation tubes of lactose broth. Incubate 24–48 hr at 35°. Streak eosin methylene blue or Endo agar plates from all lactose broth cultures showing gas production. Incubate plates 24–48 hr at 35°. Examine plates of differential media for colonies of microorganisms of coliform group. Record number of coliform bacteria/g egg material as reciprocal of highest diln showing positive confirmation on differential media.

(b) *Biochemical reaction* (*optional*).—Inoculate from colonies of coliform types of bacteria appearing on differential agar plates to agar slants, **37.003**(a) or (b). Incubate 24 hr at 35°. Purify cultures for further study. Obtain biochemical reactions of purified cultures by following tests:

Kovac test (indole production);

Me red (*M.R.*) *and Voges Proskauer* (*V.P.*) *tests;*

Koser sodium citrate test (utilization of Na citrate as sole source of C).

NOTE: Follow procedure for biochemical reactions recommended in "Standard Methods for Examination of Water and Sewage," 10th ed., 1955, of American Public Health Association.

37.008 INCIDENCE OF HEMOLYTIC STAPHYLOCOCCI AND STREPTOCOCCI—PROCEDURE

Inoculate Petri plates with 1 ml portions of suitable dilns. Pour plates with veal-infusion agar contg 5% defibrinated horse, sheep, or rabbit blood (0.5 ml blood/10 ml medium). Cool agar to 45° and add blood just prior to pouring plates. Incubate plates 24 hr at 35°. Confirm presence of coccus types of microorganisms by microscopic examination of smears taken from

representative colonies and stained by Gram method. Express final results as number/g.

37.009 TESTS FOR FUNGI—PROCEDURE

Inoculate Petri plates with 1 ml portions of suitable dilns. Pour inoculated plates with malt agar previously cooled to 42–45° and acidified to pH 3.5, **37.004(a)**. Incubate plates 5 days at 20° or at room temp., if 20° incubator is not available. Express final results as number of fungi/g egg material. Confirm yeast colonies by microscopic examination of smears stained by Gram method.

37.010 DIRECT MICROSCOPIC COUNTS—OFFICIAL

North aniline oil-methylene blue stain.—Mix 3.0 ml aniline oil with 10.0 ml alcohol, and add 1.5 ml HCl slowly with constant agitation. Add 30.0 ml satd alc. methylene blue soln, dil. to 100.0 ml with H_2O, and filter.

(a) *Liquid and frozen eggs.*—Place 0.01 ml undild egg material on clean, dry microscopic slide and spread over area of 2 sq. cm (circular area with diam. of 1.6 cm suggested). Let film prepn dry on level surface at 35–40°. Immerse in xylene not <1 min.; then immerse in alcohol not <1 min. Stain not <45 sec. in North aniline oil-methylene blue stain (10–20 min. preferred; exposure up to 2 hr does not overstain). Wash slide by repeated immersions in H_2O and dry thoroly before examination. Observe subsequent procedure and precaution as in "Standard Methods for Examination of Dairy Products," 10th ed., 1953, American Public Health Association. Express final results as number of bacteria/g egg material (double microscopic factor, since 2 sq. cm area is used).

(b) *Dried eggs.*—Place 0.01 ml of the 1–10 or 1–100 diln of dried egg material on clean, dry microscopic slide and spread over 2 sq. cm.

NOTE: 0.1*N* LiOH may be used as diluent and is preferred for samples that are relatively insol. Circular area with diam. of 1.6 cm is preferable. Addn of drop of H_2O to each film facilitates uniform spreading.

Proceed as in (a). Double microscopic factor, since area of 2 sq. cm is used, and multiply count by 10 or 100, depending on whether film was prepd from 1–10 or 1–100 diln.

SELECTED REFERENCES

(*1*) J. Assoc. Offic. Agr. Chemists **22**, 625(1939).
(*2*) Ibid. **36**, 91, 316(1953).

38. Microchemical Methods

Bromine, Chlorine, and Iodine

Carius Combustion Method (1)—Official

(Do not alter combustion conditions such as temperature, size of sample, volume of acid, etc. Variations from specified conditions present dangerous explosion hazard.)

38.001 REAGENTS

(a) *Fuming nitric acid.*—Reagent grade, halogen-free, sp. gr. 1.50.

(b) *Silver nitrate.*—Reagent grade, powd.

38.002 APPARATUS

(a) *Combustion tubes.*—Fig. 82. Use clean, heavy- or thin-wall Pyrex tubes, free from flaws,

FIG. 82.—COMBUSTION TUBE

with round seal at bottom, and with following specifications. (Vol. HNO_3 and temp. depend on combustion tube used.)

(b) *Furnace.*—Elec., to hold 4 or more tubes at ca 45° angle. Must maintain temp. of $250 \pm 10°$ or $300 \pm 10°$ for 5 hr or more, with not >5° difference between any 2 points on a tube or 5° difference between similar points on any 2 tubes. Must have variable resistor or other device to adjust furnace to desired temp. Open end of furnace wells must have safety device to retain glass in furnace in case tube explodes, and device must be provided for removing individual tubes from wells (*1*).

(c) *Filter tubes.*—Micro 3 ml filter tube with medium-coarse porosity fritted disk (av. pore diam. 15–25 μ) (*2*).

(d) *Siphon.*—Make from 3 mm o.d. glass tubing, with parallel arms, one 50 and other 250 mm long, and with 110 mm connecting section rising with 13° slope to longer arm (*2*).

38.003 SAMPLE

Using microchemical balance, weigh 5–20 mg sample contg min. of 1.5 mg Cl, 2.5 mg Br, or 3.2 mg I; or using semimicrochemical balance, weigh 10–20 mg sample contg min. of 2.5 mg Cl, 4.5 mg Br, or 5.7 mg I.

(a) *Solid samples.*—Weigh by difference in weighing tube (*2*).

(b) *Viscous liquids or gummy solids.*—Weigh in porcelain boat.

(c) *Volatile liquids.*—Weigh in 5 cm sealed glass tube, 1–2 mm i.d. with capillary tip. Break off tip of capillary before placing in combustion tube, sealed end down.

38.004 DETERMINATION

Place weighed sample in combustion tube, add powd. $AgNO_3$ 100% in excess of amount estimated to be necessary, and add 0.5 ± 0.05 or 0.3 ± 0.03 ml fuming HNO_3, depending on type of combustion tube, **38.002**(a). Using blast lamp and holding at 30–40° angle, rotate tube slowly in flame until wall thickens, pull out, and seal off narrow neck of tube. Wall of seal should be not $<\frac{3}{4}$

COMBUSTION TUBE	WALL THICKNESS MM	OUTSIDE DIAM. MM	LENGTH MM	LENGTH OF SEALED TUBE BETWEEN BOTTOM AND START OF TAPER AT SHOULDER MM	VOL HNO_3 (SP. GR. 60°F, APPROX. 1.5) ML	TEMP °C
Heavy-wall	2.3 ± 0.3	13 ± 0.8	210 ± 10	150 to 175	0.5	250
Thin-wall	1.2 ± 0.2	13 ± 0.7	240 ± 10	180 to 210	0.3	300

of thickness of tube wall and sealed tube should have length shown in table. (If sample and HNO_3 react at room temp., immediately cool bottom of tube in ice-H_2O or Dry Ice-acetone bath, remove, and seal at once.) Place tube in furnace immediately and heat 5 hr at 250 or 300 ± 10°, according to tube size.

Observe following precautions before and during opening of combustion tubes: (*a*) Place asbestos glove on hand used to hold small burner or hand torch; (*b*) protect face by transparent face mask or work behind safety shield; (*c*) be certain tube has cooled to room temp.; (*d*) force tip of tube ca 2″ out of furnace well; (*e*) gently flame end to drive all acid from tip and upper walls; and (*f*) soften tip with small hot flame until pressure in tube is released by blowing out softened glass.

Remove vented tube from furnace and cut off constricted end by scratching tube with file ca ½″ from shoulder of open end, moistening scratch, and touching with tip of very hot glass rod. Remove end of tube with care and fire polish to avoid contaminating ppt with glass splinters.

Rinse walls of tube with H_2O until tube is ca ¾ full, place in steam or boiling H_2O bath, protected from light, and digest until ppt coagulates (ca 30 min.). Longer digestion is required for I than for Br or Cl since eutectic mixt. of $AgNO_3$ and AgI is formed, which melts below temp. of steam bath and persists as heavy yellow oil on bottom of tube. Stirring with glass rod speeds up soln of $AgNO_3$ and greatly reduces digestion time, which *must* be continued until ppt is in form of *fine powder*. If excessive amounts of $AgNO_3$ have been used, greater dilns than specified are required for complete pptn. Therefore, after digestion appears complete, pipet few drops of clear supernatant aq. soln into test tube contg several ml H_2O. If turbidity occurs, entire supernatant must be dild with H_2O until pptn stops, and digestion to coagulate ppt must be repeated. If no turbidity occurs on diln, pipetted portion may be discarded.

Place previously washed, dried, and weighed filter tube in 1 hole stopper in suction flask, connect short arm of siphon tube to filter tube thru small rubber stopper, and adjust tube so that long arm of siphon almost touches ppt. Transfer ppt to filter tube by suction. Rinse tube and ppt alternately with 1% HNO_3 and alcohol, using 2 or 3 ml portions for each rinse.

Remove siphon, rinse tip and stopper with alcohol, and rinse filter tube and ppt first with the acid, then with alcohol. Wipe outside of filter tube with moist chamois (or cheesecloth) and dry 30 min. at 125° in air oven or 30 min. at 80° in vac. oven; cool to room temp. (ca 30 min.) and weigh. Handle dry tube with chamois finger cots or tweezers. Make blank detn and subtract any correction from wt sample ppt.

$$(\text{wt ppt} - \text{blank}) \times \frac{\text{Cl}}{\text{AgCl}} \times \frac{100}{\text{wt sample}} = \%\ \text{Cl}$$

$$(\text{wt ppt} - \text{blank}) \times \frac{\text{Br}}{\text{AgBr}} \times \frac{100}{\text{wt sample}} = \%\ \text{Br}$$

$$(\text{wt ppt} - \text{blank}) \times \frac{\text{I}}{\text{AgI}} \times \frac{100}{\text{wt sample}} = \%\ \text{I}.$$

Carbon and Hydrogen (*3*)—Official

38.005 REAGENTS

(**a**) *Copper oxide.*—Wire form, ca 1 mm diam. and 3–4 mm long; discard material finer than "20-mesh." Ignite 1 hr at 800–900° before placing in combustion tube.

(**b**) *Platinum gauze, 52 mesh.*—From three 3×5 cm sections, make 3 rolls, each 30 mm long ×7 mm o.d. Boil 15 min. in HNO_3 (1+1) and ignite in nonluminous Bunsen flame.

(**c**) *Asbestos.*—Gooch asbestos; ignite 30 min. at 800–900° and store in wide-mouth bottle.

(**d**) *Silver.*—Fine wire or ribbon; if tarnished, reduce in stream of H at 350–450°.

(**e**) *Lead dioxide.*—Pellets, 1–2 mm diam., special grade for microanalysis; or prep. by digesting commercial grade powder 2 hr in HNO_3, let stand 1 hr, decant, wash with H_2O until acid-free, evap. to dryness, and cut into 2 mm cubes. Roll cubes in jar to round corners and sieve out powder.

(**f**) *Glass wool.*—Pyrex, pliable.

(**g**) *Dehydrite or Anhydrone.*—($Mg(ClO_4)_2$, anhyd.) Break pieces to <3 mm long; discard portion passing thru No. 40 sieve.

(**h**) *Ascarite.*—(NaOH on asbestos.) Use commercial prepn of "8–20 mesh."

38.006 APPARATUS (*See* FIG. 83)

(**a**) *Oxygen.*—Cylinder with pressure regulator adjustable from 0–10 lb pressure on low-pressure side and with needle-valve control.

(**b**) *Preheater.*—Specifications as recommended by Committee on Microchemical Apparatus, Div. Anal. Chem. (A.C.S.) (*2*), except with 12/2 ball joint. Rubber connectors may be used.

(**c**) *Bubble counter and U-tube.*—According to recommended specifications (*2*) except with ball joints. Rubber connectors may be used.

(**d**) *Combustion tube.*—Fused quartz (or Vycor) glass, dimensions according to recommended specifications but with 12/2 ball joint on side arm and 5/12 or 7/15 inner joint on exit end. Rubber connectors may be used. (Pyrex tubes may be used but furnace temps should not be >725°.)

(**e**) *Absorption tubes.*—Pregl type, according to recommended specifications (*2*) but with 5/12 joints; alternatively, Prater type, semimicro size

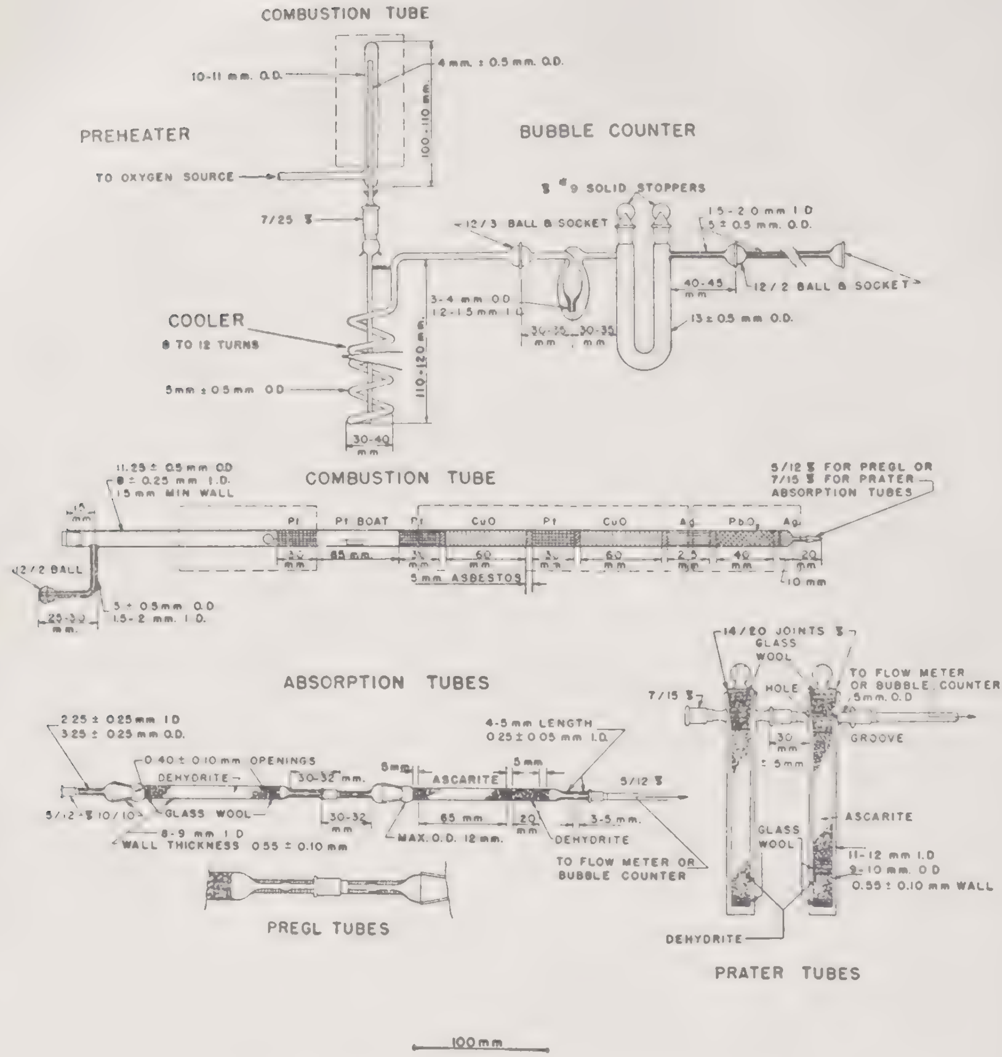

FIG. 83.—CARBON AND HYDROGEN APPARATUS

with 7/15 joints. Rubber connectors may be used.

(f) *Bubble counter or flowmeter.*—Any convenient arrangement to measure 10–30 ml/min. gas flow from exit end of second absorption tube.

(g) *Preheater furnace.*—Elec., 12–14 mm i.d. ×5″ long, maintained at 600±25°. Gas heaters may be used for all furnaces but specified temps should be maintained. Temps of furnaces are measured at center of furnace inside empty combustion tube with one end stoppered.

(h) *Burning furnace.*—Elec., 13–14 mm i.d. ×4″ long. Furnace should reach 600–700° in 5 min., ca 800° in 15 min., with max. of 850° in 30 min. *See* (g).

(i) *Long furnace.*—Elec., 13–14 mm i.d. ×8″ long; maintained at 775–800°. *See* (g).

(j) *Constant temperature mortar.*—Elec., 13–14 mm i.d. ×3″ long, thermostatically controlled at 177±2°. *See* (g).

38.007 PREPARATION OF APPARATUS

(a) *Preheater.*—Place CuO in preheater tube, connect spiral cooling coil, immerse coil in beaker of H_2O, and support assembly by suitable clamps and stand. Place furnace over preheater tube and maintain at ca 600°. Connect side arm of combustion unit to needle valve of O pressure regulator by suitable tubing, rubber or Tygon.

(b) *Bubble counter-U-tube.*—Fill bubble counter and U-tube by placing glass wool plug at bottom of U, fill side next to bubble counter with Dehydrite to within ½″ of side arm, and cap with another glass wool plug. Place Ascarite layer in other side to within 1½″ of side arm; then insert glass wool plug, ca 1″ of Dehydrite, and finally second plug. Cement in stoppers with glass cement or paraffin; then with medicine dropper add H_2SO_4 to bubbler until level is 3–4 mm above bubbler tip. Connect to preheater with pressure clamp.

(c) *Combustion tube.*—Clean and dry combustion tube. Place 10 mm roll of Ag in exit end with 1 or 2 strands reaching to open end of ground joint. Insert loose asbestos plug (not choking plug), 40 mm PbO_2, asbestos plug, and second Ag roll 25 mm long, which should extend into long

furnace about ½″. Insert asbestos plug, 60 mm CuO, asbestos plug, 30 mm Pt gauze roll, asbestos plug, 60 mm CuO, asbestos plug, and finally 30 mm Pt gauze, which should extend about 10 mm beyond end of long furnace. Place prepd tube in furnaces with exit end protruding far enough beyond constant-temp. mortar to permit connecting absorption tubes. Connect side arm to bubble counter-U-tube.

(d) *Absorption tube.*—Place glass wool plug in end of H_2O absorption tube, fill tube to within ½″ of other end with Dehydrite or Anhydrone, and cap with second glass wool plug. If Pregl tubes are used, seal ground-glass joint with enough glass cement to give clear seal, and remove any excess on outer surface of tube with cotton dipped in benzene or other solvent. If Prater tubes are used, lubricate lower ⅔ of inner joint with min. of light stopcock grease and insert in outer tube.

Prep. CO_2 absorption tube by placing glass wool plug in end and fill tube to ca 1½″ of other end with Ascarite. Insert ¼″ glass wool plug, add ¾″ layer of Dehydrite, and cap filling with another glass wool plug. Complete assembly of absorption tube as for H_2O absorption tube. Connect absorption tubes to combustion tube with ground joints (use no lubricant) or with special impregnated rubber tubing.

Attach calibrated bubble counter or flowmeter to exit end of CO_2 absorption tube.

38.008 DETERMINATION

(a) *Conditioning apparatus.*—Condition prepd and assembled app. by heating combustion tube 3–4 hr with long furnace at 775–800° and with O flowing thru app. at rate of 15–20 ml/min. Use 3–4 lb O pressure on low pressure side of regulator. At the same time, make 2 simulated sample burnings, without sample, with burning furnace at 825–850°. (Temp. must be ca 100° lower if Pyrex combustion tubes are used.)

Burn unweighed 10–15 mg sample to condition combustion and absorption tubes. With absorption tubes connected, adjust needle valve on regulator so that O flow is 15–20 ml/min. and place burning furnace ca 3″ from long furnace. Place micro Pt boat contg sample in combustion tube ca 2″ from long furnace. Insert third Pt roll 1″ from boat, and stopper tube. Turn on burning furnace and let it reach ca 600° before starting sample combustion by moving furnace over sample at rate of 1″ in 6–8 min. Move burning furnace across sample only once, taking 18–24 min. for full travel of furnace. Turn off burning furnace 5 min. after it reaches long furnace but continue to sweep O thru tube for addnl 15 min. before disconnecting absorption tubes.

Remove absorption tubes and place by balance to equilibrate. Handle tubes only with clean chamois finger cots. If Prater tubes are used, turn joints ¼ turn to seal. If rubber connections are used, wipe only tips of tubes with moist, then dry, chamois before placing them by balance. Wait 10 min. if ground joints were used or 15 min. if rubber connections were made; then weigh CO_2 absorption tube first and H_2O absorption tube next, using glass tare with vol. and surface ca equal to that of absorption tubes. Record wts of tubes and reconnect to combustion tube for subsequent analysis.

(b) *Proving the apparatus.*—Replace boat with one contg 10–15 mg sample of std compound such as NBS microchemical std, weighed to nearest 0.01 mg. Repeat combustion and weighing procedure as in (a). Calc. % C and H in std sample from increase in wt of CO_2 and H_2O absorption tubes. Repeat analysis until results from 2 consecutive runs are within 0.30% of theoretical values and means of C and H results are within 0.20% of theoretical value for the std compound. (Humidity conditions of room may make it necessary to correct apparent wt of H_2O by subtracting a blank value.)

When app. meets this test, analyses of samples are made as above.

Nitrogen (*4*)

Micro-Kjeldahl Method—First Action

(Not applicable to material containing N–N or N–O linkages)

38.009 REAGENTS

(a) *Sulfuric acid.*—Sp. gr. 1.84, N-free.

(b) *Mercuric oxide.*—N-free.

(c) *Potassium sulfate.*—N-free.

(d) *Sodium hydroxide-sodium thiosulfate soln.*—Dissolve 60 g NaOH and 5 g $Na_2S_2O_3.5H_2O$ in H_2O and dil. to 100 ml or add 25 ml of 25% $Na_2S_2O_3.5H_2O$ to 100 ml of 50% NaOH soln.

(e) *Boric acid soln.*—Satd soln.

(f) *Indicator soln.*—(1) *Methyl red-methylene blue.*—Mix 2 parts 0.2% alc. Me red soln with 1 part 0.2% alc. methylene blue soln; or (2) *Methyl red-bromocresol green soln.*—Mix 1 part 0.2% alc. Me red soln with 5 parts 0.2% alc. bromocresol green soln.

(g) *Hydrochloric acid.*—0.02*N*. Prep. as in **42.009** and stdze as in **42.013** or **42.015**.

38.010 APPARATUS

(a) *Digestion rack.*—Use rack with either gas or elec. heaters which will supply enough heat to 30 ml flask to cause 15 ml H_2O at 25° to come to rolling boil in not <2 or >3 min.

(b) *Distillation apparatus.*—Use one-piece or Parnas-Wagner distn app. recommended by Committee on Microchemical Apparatus, ACS (*5*).

(c) *Digestion flasks.*—Use 30 ml regular Kjeldahl or Solty's type flasks (*5*). For small samples, 10 ml Kjeldahl flasks may be used.

38.011 DETERMINATION

Weigh sample requiring 3–10 ml 0.01 or 0.02N HCl and transfer to 30 ml digestion flask. If sample wt is <10 mg, use micro balance. Wt should be not >100 mg dry org. matter. Use charging tube for dry solids, porcelain boat for sticky solids or non-volatile liquids, and capillary or capsule for volatile liquids. Add 1.9 ± 0.1 g K_2SO_4, 40 ± 10 mg HgO, and 2.0 ± 0.1 ml H_2SO_4. If sample wt is >15 mg, add addnl 0.1 ml H_2SO_4 for each 10 mg dry org. matter >15 mg. Make certain that acid has sp. gr. of at least 1.84 if sample contains nitriles. (10 ml flasks and ½ quantities of reagents may be used for samples <7 mg.) Add boiling chips which pass No. 10 sieve. If boiling time for digestion rack heaters is 2–2.5 min., digest 1 hr after all H_2O is distilled and acid comes to true boil; if boiling time is 2.5–3 min., digest 1.5 hr. (Digest 0.5 hr if sample is known to contain no refractory ring N.)

Cool, add min. quantity of H_2O to dissolve solids, cool, and place thin film of Vaseline on rim of flask. Transfer digest and boiling chips to distn app. and rinse flask 5 or 6 times with 1–2 ml portions of H_2O. Place 125 ml Phillips beaker or erlenmeyer contg 5 ml satd H_3BO_3 soln and 2–4 drops indicator under condenser with tip extending below surface of soln. Add 8–10 ml $NaOH\text{-}Na_2S_2O_3$ soln to still, collect ca 15 ml distillate, and dil. to ca 50 ml. (Use 2.5 ml H_3BO_3 and 1–2 drops indicator, and dil. to ca 25 ml if 0.01N HCl is to be used.) Titr. to gray end point or first appearance of violet. Make blank detn and calc. % N = [(ml HCl − ml blank) × normality × 14.008 × 100]/mg sample.

Phosphorus (*6*)—Official

38.012 REAGENTS

(**a**) *Nitric-sulfuric acid mixture.*—Slowly pour 420 ml HNO_3 into 580 ml H_2O; then slowly add 30 ml H_2SO_4.

(**b**) *Ammonium nitrate soln.*—2%. Prep. 2% soln of NH_4NO_3 in H_2O, add 2 drops HNO_3, and store in g-s. bottle. Filter immediately before use.

(**c**) *Molybdate reagent.*—Dissolve 150 g powd. NH_4 molybdate in 400 ml H_2O and cool under tap. Place 50 g $(NH_4)_2SO_4$ in 1 L vol. flask, dissolve in mixt. of 105 ml H_2O and 395 ml HNO_3, and cool under tap. Pour cooled molybdate soln slowly into $(NH_4)_2SO_4$ soln with constant stirring and cooling under tap. Dil. soln to 1 L, store in refrigerator 3 days, filter, and store in paraffin-lined, g-s., brown bottle in refrigerator. Filter reagent immediately before use and check by periodically analyzing std sample.

38.013 APPARATUS

(**a**) *Kjeldahl digestion flasks (30 ml), rack, and manifold.*—See 38.010(**a**) and (**c**).

(**b**) *Filter tubes and filtration assembly.*—See 38.002(**c**) and (**d**).

(**c**) *Rubber stoppers.*—Two or three small, solid rubber stoppers to loosen ppt from walls of flask.

38.014 DETERMINATION

Weigh 3–20 mg sample, depending on P content and whether micro or semimicro balance is used (max. wt ppt = 50 mg). Weigh in charging tube, if possible, and transfer to Kjeldahl flask. Use porcelain boat for sticky solids and viscous liquids, and glass capillary for volatile liquids.

Add 0.5 ml H_2SO_4 followed by 4–5 drops HNO_3. Heat on digestion rack to white SO_3 fumes and cool under tap. Add 4–5 drops HNO_3, repeat digestion, and cool under tap. Add 4–5 drops HNO_3 and again digest to SO_3 fumes. Cool to room temp.; add 2 ml acid mixt., 38.012(**a**), and 12.5 ml H_2O, rinsing down neck of flask. (If porcelain boat was used to add sample, remove boat with Pt wire; if glass capillary was used, filter digestion mixt. to remove capillary. Rinse filter and boat or capillary with 12.5 ml H_2O used to dil. sample.)

Place flask on steam bath 15 min. to convert P to H_3PO_4. Remove from steam bath and pipet 15 ml molybdate reagent, 38.012(**c**), into center of digest, not down walls of flask. Let stand 2–3 min.; then gently swirl to mix contents, being careful to prevent reagents from splashing on neck of flask. Cover flask and set in dark place overnight.

Condition filter tube as described below and weigh empty tube. Connect tared filter tube to filtration assembly and transfer ppt to filter thru siphon tube. Wash flask alternately with 1–2 ml portions of the NH_4NO_3 soln and alcohol. Add 2–3 small rubber stoppers to digestion flask, shake to loosen any ppt, and transfer with the NH_4NO_3 soln and alcohol. Disconnect siphon tube; rinse ppt from tip and stopper into filter tube with the NH_4NO_3 soln and alcohol. Wash ppt with more NH_4NO_3 soln, alcohol, and finally with acetone, and suck dry. Wipe filter tube with chamois skin, place in vertical position in vac. desiccator contg no desiccant, and evacuate to 1 mm for 30 min. with mechanical vac. pump in continuous operation. Release vac. and weigh *immediately* to nearest 0.1 mg. (Rapid weighing is essential because of hygroscopic nature of ppt.)

Mg ppt × 0.014524 × 100/mg sample = %P.

Sulfur

Titrimetric Carius Combustion Method (7)—Official

(Not applicable in presence of P)

38.015 REAGENTS

(**a**) *Fuming nitric acid.*—Reagent grade, sp. gr. 1.50.

(b) *Sodium chloride.*—Reagent grade, fine crystals.

(c) *Barium chloride soln.*—Approx. 0.02*N*. Stdze by titrg 5–7 mg freshly dried K_2SO_4, ACS (weighed to nearest 0.01 mg), by method used for sample titrn. Correct titrn for indicator error by blank detn.

(d) *Potassium sulfate.*—ACS, powd. and dried.

(e) *Phenolphthalein soln.*—0.5% soln in 50% alcohol.

(f) *Sulfate indicator.*—"T.H.Q." sulfate indicator (Betz Laboratories, Philadelphia, Pa.) or mix 0.1 g K rhodizonate with 15 g sucrose by grinding in mortar.

38.016 APPARATUS

(a) *Combustion tubes and furnace.*—*See* **38.002**(a) and (b).

(b) *Titration assembly.*—5 ml buret graduated in 0.01 ml; rectangular titrn cell ca 2×4×5 cm with min. capacity of 50 ml; and std orange-red glass color filter (Corning No. 3482, lantern shade yellow) selected to have 37% transmittance at 550 mμ. Place cell and filter side by side on milk glass window illuminated from below, preferably by fluorescent light. Mask light source so that only cells and filter are illuminated.

38.017 SAMPLE

Using microchemical balance, weigh 5–20 mg sample contg not <0.75 mg S, or using semimicrochemical balance, weigh 10–20 mg sample contg not <0.75 mg S (1.5 mg for gravimetric detn). Weigh samples as in **38.003.**

38.018 DETERMINATION

Place weighed sample in combustion tube, add NaCl 100% in excess of amount equiv. to S in sample, and proceed as in **38.004,** beginning "and add . . . fuming HNO_3 . . . " thru end of third par. " . . . with glass splinters."

Transfer contents of tube to 50 ml beaker, rinsing tube 4–6 times with 3–5 ml portions H_2O. Evap. to dryness on steam bath.

Dissolve residue in 10 ml H_2O, pour soln into titrn cell, add 1 drop phthln, and make just alk. with ca 0.1*N* NaOH, then acid with ca 0.02*N* HCl, adding 1 drop excess. Add ca 0.15 g of the sulfate indicator, stir to dissolve, and rinse beaker 2 or 3 times, using enough alcohol so that final soln contains ca 50%. Titr. with the std $BaCl_2$ soln from 5 ml buret until stable color of soln immediately after stirring matches std glass color filter. Make certain end point taken is real and not pseudo end point which fades on standing 1–2 min. Det. blank on reagents and correct titrn.

$$(\text{ml } BaCl_2 - \text{ml blank}) \times \text{normality} \times 16.033 \times 100/\text{wt sample (mg)} = \% \text{ S}$$

Gravimetric Carius Combustion Method (8)—Official

(Applicable in presence of P)

38.019 APPARATUS

Crucible and filter stick.—Porcelain crucible, ca 15 ml capacity, with black inside glaze, wt ca 10 g; with porcelain filter stick, with unglazed bottom, wt ca 2 g (*2*).

38.020 DETERMINATION

Dissolve residue, **38.018,** in 3 ml H_2O, pour into previously ignited and weighed (with filter stick) porcelain crucible, and rinse beaker with four 2 ml portions H_2O. Place crucible on steam bath until soln is near b.p. If vol. exceeds 10–11 ml, evap. to this vol. Add dropwise 0.5 ml 10% $BaCl_2$ soln (1 ml for samples contg >5 mg S), digest at least 15 min., and cool 15 min.

Connect porcelain filter, previously ignited and weighed with crucible, to arm of siphon with rubber tubing. Connect other arm of siphon to suction flask thru rubber stopper. Lower filter into crucible, slowly draw off soln, and rinse ppt, walls of crucible, and filter with five or six 3 ml portions HCl (1+300), drawing off as much liquid as possible. Carefully detach filter, place in crucible, wipe outside of crucible and end of filter with moist chamois or cheesecloth, and handle thereafter with crucible tongs. Place crucible and filter in larger crucible and dry in oven 10 min. at ca 110°. Ignite in muffle 10 min. at 700–750° (ppt may also be ignited by heating larger crucible contg crucible and filter to dull red heat with Meker burner), cool on metal block 30 min. or in desiccator 1 hr, and weigh. Det. blank on reagents.

$$(\text{wt } BaSO_4 - \text{blank}) \times 0.1374 \times 100/\text{wt sample} = \% \text{ S}$$

Titrimetric Catalytic Combustion Method (7)—Official

(Not applicable in presence of P)

38.021 REAGENTS

Use reagents **38.015**(c), (d), (e), and (f), and satd Br-H_2O stored in g-s. bottle.

38.022 APPARATUS

(a) *Oxygen supply.*—Use O pressure cylinder with 2 stage reducing valve which has needle valve control on low pressure side, or other source which will supply pure O at 12–15 ml/min.

(b) *Purification train.*—If O is not free from S-contg gases, purify by passing thru tube contg first Dehydrite, then Ascarite.

(c) *Combustion tube.*—Quartz (or Vycor) with dimensions shown in Fig. 84.

(d) *Absorber.*—Beasley type spiral connected to combustion tube by ground joint.

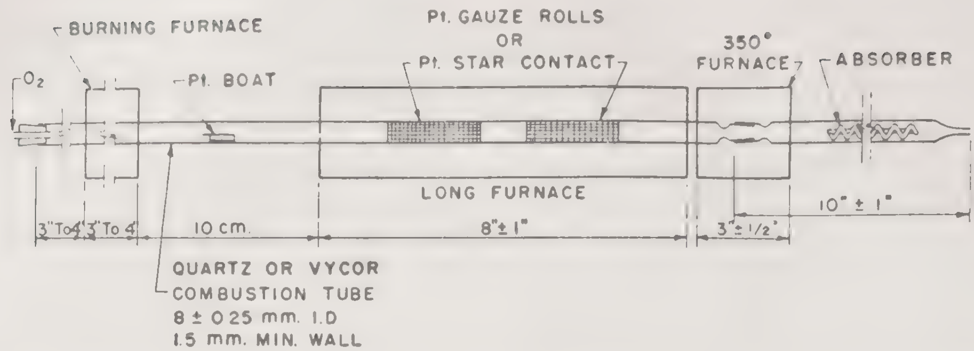

FIG. 84.—COMBUSTION APPARATUS FOR SULFUR DETERMINATION BY CATALYTIC METHOD

(e) *Catalyst.*—2 Pt star contacts (*2*) or 2 Pt gauze rolls made from 5 cm squares of ca 50 mesh gauze with diam. within 1 mm of i.d. of combustion tube.

(f) *Furnaces.*—Elec. (preferred) or gas, both providing temp. inside combustion tube of at least 750°, preferably 800° or higher. Operate sample burner manually, or preferably mechanically, with rate of motion of 0.5 cm/min. Operate short furnace for ground joint, preferably elec., at ca 350°.

(g) *Titration assembly.*—See **38.016(b)**.

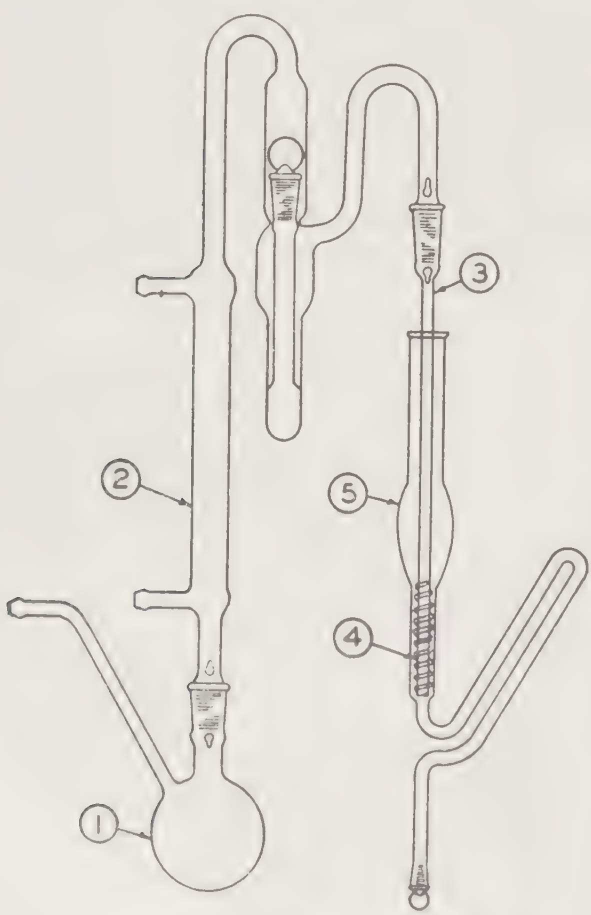

FIG. 85.—MODIFIED CLARK APPARATUS

38.023 SAMPLE

Use sample as in **38.017.**

38.024 DETERMINATION

Clean catalysts by boiling 10 min. in ca 6*N* HNO_3 and flaming over non-luminous flame. Place catalysts in combustion tube and set tube in furnaces as shown in Fig. 84; heat long furnace to at least 750° and preferably 800° or higher.

Moisten entire spiral of absorber by drawing into it, with gentle suction, 5–10 ml Br-H_2O. Care must be exercised to keep ground joint dry. Drain excess soln from absorber and attach to combustion tube with ground joint in 350° furnace. Place sample in combustion tube 5 cm from long furnace, connect O source, and adjust flow to 12–15 ml/min., using flowmeter or calibrated bubble counter.

Heat sample burner to at least 750° (preferably 800° or higher), bring to 5 cm from sample, and move over sample area at rate of 0.5 cm/min. (burning time 20 min.). Continue sweeping with O for 10 min. (total combustion time 30 min.). Disconnect absorber and let joint cool 3–5 min.

Rinse contents quantitatively into 50 ml erlenmeyer, using ca 15 ml H_2O. Rinse outside of absorber tip. Add 5 drops Br-H_2O, boil until Br is expelled, and cool under tap. Continue as in **38.018,** beginning "pour soln into titrn cell . . . "

38.025 *Gravimetric Catalytic Combustion Method (8)—Official*

(Applicable in presence of P)

Rinse contents of absorber, **38.024,** quantitatively into previously ignited and weighed (with filter stick) porcelain crucible, **38.019,** using five 2 ml portions H_2O. Continue as in **38.020,** beginning "Place crucible on steam bath . . . "

Alkoxyl Groups (*9*)—Official

38.026 REAGENTS

(a) *Acetic acid-potassium acetate-bromine soln.*—Dissolve 10 g KOAc in enough HOAc to make 100 ml, and add 3 ml Br.

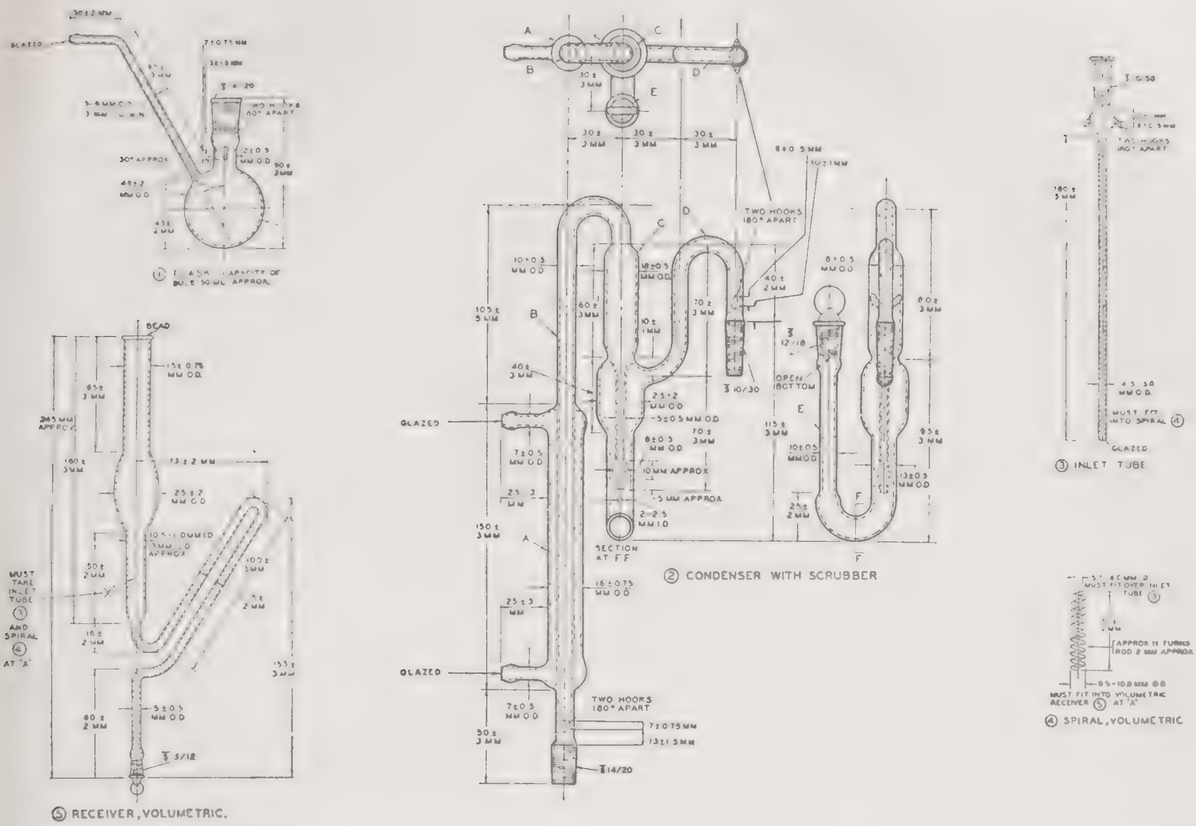

FIG. 86.—DETAILS OF MODIFIED CLARK APPARATUS

(b) *Sodium acetate soln.*—Dissolve 25 g NaOAc $.3H_2O$ in enough H_2O to make 100 ml.

(c) *Starch indicator.*—Mix ca 2 g finely powdered potato starch with cold H_2O to thin paste; add ca 200 ml boiling H_2O, stirring constantly, and immediately discontinue heating. Add ca 1 ml Hg, shake, and let soln stand over the Hg.

(d) *Sodium thiosulfate std soln.*—0.02*N*. Bring 1 L distd H_2O to boil to remove CO_2, and cool while loosely covered. Dissolve 4.96 g $Na_2S_2O_3$ $.5H_2O$ in the H_2O and dil. to 1 L. Transfer to brown, rubber-stoppered bottle. Add 1 ml $CHCl_3$ as preservative and shake few min. Rubber stopper absorbs $CHCl_3$, increasing efficiency of preservative. Stdze monthly if pool of $CHCl_3$ is present; if pool is absent, stdze every few days.

(e) *Hydriodic acid.*—Place 250 ml constant boiling (57%) HI (sp. gr. 1.7) in 500 ml round-bottom flask connected by ₮ joint to air condenser, and reflux 2 hr while stream of CO_2 or N bubbles thru from glass tube extending to bottom. Do not let acid vapors come in contact with org. material. As soon as refluxing stops, discontinue gas flow. Cool, and store in g-s. bottle.

38.027 STANDARDIZATION OF SODIUM THIOSULFATE SOLUTION

Accurately weigh ca 5 mg $KH(IO_3)_2$, using porcelain boat or charging tube, transfer to 125 ml g-s. erlenmeyer, and dissolve in 10 ml H_2O. Add 3 ml HCl and 2 ml *freshly prepd* 4% KI soln, and stopper. After 2 min., dil. with H_2O to 40 ml and titr. liberated I with the $Na_2S_2O_3$ soln. When I color almost disappears, add several drops starch indicator. Continue titrn until blue has been converted to faint pink end point.

$$\text{Normality} = \text{mg } KH(IO_3)_2 \times 0.03077/\text{ml } Na_2S_2O_3.$$

38.028 APPARATUS

Use modified Clark app., Figs. 85 and 86.

38.029 DETERMINATION

Fill scrubber half way with the NaOAc soln, and fill receiver $\frac{2}{3}$ full with freshly prepd KOAc-Br soln. Weigh enough sample in Pt boat to require ca 8 ml $Na_2S_2O_3$ soln in detn, and place in bottom of boiling flask. Add 2.5 ml melted phenol from wide-tip pipet and 5 ml of the HI, and connect boiling flask. Pass CO_2 thru app. from side arm of flask at uniform rate of 15 ml/min. Let reaction mixt. remain at room temp. 30 min. With manteled micro burner, boil liquid at such rate that vapors of boiling liquid rise into condenser,

but not more than half way; continue boiling 60 min. (first 30 min. with H_2O circulating thru condenser and last 30 min. with H_2O drained from condenser). Disconnect flask, remove receiver, and rinse delivery tube and contents of receiver into 125 ml erlenmeyer contg 5 ml of the NaOAc soln. Adjust vol. to ca 50 ml and add *formic acid* dropwise until excess Br is destroyed.

Remove any Br vapors by blowing air over liquid; then add 0.5 g KI and 5 ml 10% H_2SO_4. Swirl soln to dissolve KI and mix contents; then titr. liberated I with the $Na_2S_2O_3$ soln, using starch indicator as in stdzn.

Det. blank on all reagents by making detn without sample and calc. % alkoxyl group as follows:

(ml in detn − ml in blank) × normality × equiv. wt × 100/sample wt in mg = % alkoxyl group

Equiv. wt: methoxyl = 5.17; ethoxyl = 7.51.

SELECTED REFERENCES

(*1*) J. Assoc. Offic. Agr. Chemists **35**, 291 (1952); **36**, 91, 319(1953); **40**, 381(1957); **41**, 297 (1958); Anal. Chem. **23**, 1689(1951).

(*2*) Anal. Chem. **21**, 1555(1949).

(*3*) J. Assoc. Offic. Agr. Chemists **32**, 561 (1949); **34**, 94, 607(1951); Anal. Chem. **23**, 911 (1951).

(*4*) J. Assoc. Offic. Agr. Chemists **32**, 561 (1949); **33**, 179(1950); **43**, 689(1960).

(*5*) Anal. Chem. **23**, 523(1951).

(*6*) J. Assoc. Offic. Agr. Chemists **40**, 386 (1957).

(*7*) Ibid. **35**, 305(1952); **36**, 91, 335(1953).

(*8*) Ibid. **38**, 377(1955).

(*9*) Ibid. **39**, 401(1956).

39. Nutritional Adjuncts

CHEMICAL METHODS

Vitamin A in Margarine (*1*)—First Action

39.001 PRINCIPLES

Unsaponifiable portion of margarine is chromatographed on adsorption column consisting of 2 segments of activated and stdzd Al_2O_3 sepd by middle segment of alk. Al_2O_3. If eluate fraction contg vitamin A is colored, it must be further chromatographed on column of MgO. Top segment of Al_2O_3 column prevents caking, and initiates sepn of vitamin A from carotene and other interfering substances. Middle section of alk. Al_2O_3 seps persistent interference that cannot be sepd by other adsorbents. Final portion of column is non-fluorescent and provides suitable background for observing vitamin A fluorescence on column, facilitating control of the chromatography.

Carotene elutes from column first, second fraction of eluate is discarded, and third fraction contains vitamin A. Better control of chromatography than is possible by observing colored and fluorescent bands on column is achieved by observing fluorescence in 1 ml portions from final parts of fluorescent and colored eluates. This technic achieves sepn of vitamin A from impurities that cause erroneously high values. Adequacy of sepn is detd from ratio of absorbances of chromatographed vitamin A soln at 310 and 325 mμ.

If vitamin A eluate from Al_2O_3 column is colored, chromatography thru MgO column is necessary to prep. soln suitable for final spectrophotometric measurement. Absorbance of sample solution at 325 mμ multiplied by factor 18.3 gives concn of vitamin A in units/ml.

39.002 APPARATUS

(**a**) *Spectrophotometer and cells.*—Ultraviolet spectrophotometer with suitable source of ultraviolet light is required. (Incandescent lamp is *not* suitable source.) Spectrophotometer (such as Beckman DU or equiv.) equipped with continuous spectrum source and reading to 200 mμ is recommended. Matched quartz cells with 1.0 cm internal light path are preferable. If cells are not matched, suitable corrections must be made. Check wavelength and absorbance scales of spectrophotometer periodically (*See* Definitions of Terms and Explanatory Notes, p. xviii).

(**b**) *Chromatographic tubes.*—(1) Tube 12 mm o.d. and 9 cm long with funnel on upper end and stem on lower end 8 mm in diam. and 1.5″ long with sealed-in disk of medium porosity. (Available from Scientific Glass Products, 49 Ackerman St., Bloomfield, N.J., No. G-9855.) (2) Tube 6 mm i.d. and 25 cm long with lower 5 cm pulled out to form tapered constricted exit 2 mm i.d. Plug ca 1 cm of upper part of constricted section with glass wool. Fuse flared tube, 18 mm diam. and 14 cm long, to top of 6 mm section.

(**c**) *Vacuum gauge with bleeder valve or pinchcock regulator.*—Use vac. micro bell jar large enough to hold 100 ml beaker or flask for applying vac. and collecting eluates. Control vac. from line or H_2O aspirator by gauge and stopcock or screw clamp bleeder on T-tube.

(**d**) *Long wavelength ultraviolet lamp.*—Use lamp source of *weak* ultraviolet for observing fluorescent bands on chromatographic columns. Lamp should provide radiation in long (300 mμ) wavelength region. Suitable lamp may be built or is commercially available (Fisher Scientific Co., 11-980-40, 3600 Å wavelength; or *J. Assoc. Offic. Agr. Chemists* **28**, 76(1945)). With commercial lamps, narrow aperture or screen may be necessary to reduce amount of destructive radiation. (Vitamin A is readily destroyed by too intense ultraviolet light.)

39.003 REAGENTS

(**a**) *Potassium hydroxide soln.*—50% by wt (780 g/L).

(**b**) *Alcohol.*—Absolute and 95%. Shall not show absorbance >0.05 when measured at 300 mμ in suitable spectrophotometer in 1.0 cm quartz cell against H_2O. Isopropyl alcohol USP of same spectral purity may be substituted for absolute alcohol in absorbance measurements.

(**c**) *Ethyl ether.*—Peroxide-free. Use USP, freshly distilled, discarding first and last 10% of distillate; or use USP anesthesia grade in 0.5 lb cans. Must meet requirement for spectral purity described for alcohol, (**b**).

(**d**) *Petroleum ether.*—B.p. 30–60°, ACS, free from fluorescence and having transmission at 300 mμ >85% when measured against air in quartz spectrophotometer fitted with 1 cm cell. This solvent, available in 5 lb cans, should be suitable for chromatographic purposes. Also, in adsorbent activity test, eluent effect of 10 ml of petr. ether

by itself must cause movement of visible color no >1 cm below surface of column. To meet these requirements, purification may be necessary by adsorption and/or distillation.

(e) *Eluting solns.*—(1) 16% redistd ether in petr. ether; (2) 25% redistd ether in petr. ether; (3) 10% absolute alcohol in petr. ether. Store (1) and (2) over bright Cu strip or turnings to inhibit peroxide formation.

(f) *Sodium sulfate.*—Anhyd., granular; 10% soln must *not* be acid (red) to Me red. Must not adsorb vitamin A.

(g) *Alumina.*—Alcoa grade F-20 or Fisher "Alumina, Adsorption," Catalog No. A540. Before working with the alumina it is essential to det. that following specifications for particle size have been met: Not >50% of Al_2O_3 should pass No. 160 sieve, ca 50% should pass No. 100 sieve, but not No. 160 sieve. Remainder (not >20%) which does not pass thru No. 100 sieve, should pass No. 60 sieve. Blend thoroly before use.

(h) *Standardized alumina.*—Heat portion of Al_2O_3 3 hr in muffle at 600°, and after partial cooling, place in tightly closed screw-cap glass jar. Cool to room temp., pass thru No. 80 sieve, weigh, and place in tared screw-cap glass jar of such size that only ⅔ of vol. is used. Add H_2O dropwise, with frequent shaking of capped bottle, until Al_2O_3 contains 3% by wt of added H_2O. (Proportion of H_2O required may vary from 2 to 4%; 3% is usually sufficient for new Al_2O_3 and 2% for rejuvenated material.) Continue shaking at least 15 min. until no lumps remain and material is uniform.

Det. adsorption index as in **39.004**, after Al_2O_3 has remained in tightly capped jar overnight. (Since change in moisture content will affect adsorptivity of reagent, container must be kept tightly closed, except while removing portion of contents for use.) Adsorption index of stored material decreases with time and should be checked periodically. Al_2O_3 suitable for chromatography has adsorption index of 30–40; extremely retentive Al_2O_3 with index >50 will not permit clean-cut sepns. When index is <10 adsorbent has lost most of its retentiveness. Decreased retentiveness may be due to excess H_2O content or to changed physical state caused by overheating.

(i) *Standardized alkaline alumina.*—Mix in evapg dish portion of Al_2O_3, (g), with equal wt of 10% (w/w) aq. KOH soln. Decant excess liquid, and dry moist Al_2O_3 overnight at 100°. Pass dry material thru No. 60 sieve and place in capped bottle filled not more than ⅔ full. Add H_2O dropwise with frequent shaking until Al_2O_3 contains 3% by wt of added H_2O. Det. adsorption index as in **39.004**. To be suitable for use, alk. Al_2O_3 should have index of 7–12.

(j) *Standardized magnesium oxide.*—(Westvaco SeaSorb 43, Fisher Scientific Co.) Heat portion of MgO 4 hr at 600°. After cooling, mix with equal portion of Hyflo Super-Cel (Celite), in ½ full, tightly closed jar. Det. adsorption index as in **39.005**. To be suitable for use, the MgO-Celite mixt. should have index of 20–35.

(k) *Ext. D&C Yellow No. 10 soln.*—Dissolve 20 mg dye (Yellow OB; formerly FD&C Yellow No. 4) in 1 L petr. ether.

39.004 Determination of Adsorption Index of Alumina

Place adsorbent to be tested in chromatographic tube 6 mm i.d. and 22 cm long, contg glass wool plug at bottom. Tap material into settled position, making column 10 cm high, and attach to vac. controlled bell jar. Add to top of column 1.0 ml of Ext. D&C Yellow No. 10 soln. From accurately filled 50 ml graduated cylinder add small portions of eluting soln, 16% ether in petr. ether. Apply 5″ of vac. Det. accurately vol. of eluent required to elute dye completely from column. This vol. in ml is adsorption index. To facilitate recognition of end point, collect eluate until all apparent color on column has been removed, and then collect 2 ml fractions in small beakers until colorless fraction is obtained.

39.005 Determination of Adsorption Index of Magnesia

Place MgO-Celite mixt. to be tested in chromatographic tube 1.0–1.2 cm diam., 9 cm long, and fitted with sealed-in fritted glass disk. Apply 25″ vac. and, with aid of tamper of suitable diam., pack column tightly to height of 1.5 cm. Release vac., and add 1.0 ml of Ext. D&C Yellow No. 10 soln. From accurately filled 50 ml graduated cylinder add small (ca 2 ml) portions of eluting soln, 10% absolute alcohol in petr. ether. Apply 20″ of vac. and continue to add portions of eluent until most of color is eluted. Collect final eluates in 1.0 ml portions under 5″ of vac. Vol. in ml of eluent required to produce first colorless 1 ml fraction is adsorption index of MgO-Celite mixt. (Removal of individual fractions is easily accomplished at 5″ of vac. by slipping edge of micro bell jar over edge of its base plate.)

39.006 Sampling

Store sample in refrigerator. Remove outer layers from 1 lb prints and take sample from interior. Remove end slices from ¼ lb prints and take sample from remainder.

39.007 Determination

(a) *General precautions.*—Protect vitamin A from strong illumination by working in subdued light or by using non-actinic glassware. Avoid

undue exposure of vitamin A solns to air. Complete all steps as promptly as possible.

(b) *Saponification.*—Weigh 10 ± 0.1 g sample into 300 ml beaker, and add 75 ml of 95% alcohol and 25 ml of 50% KOH soln. Heat on elec. hot plate and stir so as to break up lumps and completely disperse sample. Maintain soln at vigorous boil 5 min. Remove heat and let stand at room temp. 20 min. with occasional stirring. Avoid rapid cooling.

(c) *Extraction.*—Transfer soln to 500 ml separator. Rinse saponification beaker with 100 ml H_2O in several portions and add rinsings to separator. Add 100 ml ether, shake vigorously, and let stand ca 2 min. Transfer aq. portion into another 500 ml separator and ext. with four 50 ml portions ether. (In case of slow sepn, add 2–5 ml 95% alcohol and swirl gently.) Combine ether exts, pour two 100 ml portions H_2O into combined ether exts, swirl gently, and sep. Ext. these 2 rinses with two 50 ml portions ether, adding ether to original ether exts. Pour two 100 ml portions H_2O thru combined ether exts and discard each washing without shaking. Then add ca 10 ml $0.02N$ KOH, shake vigorously, and discard after sepn. Rinse with successive 50 ml portions H_2O, with gentle agitation, until rinse H_2O is alkali-free to phthln. Let ether soln stand 5 min., discard sepd H_2O, transfer with rinsing to 400–500 ml tall beaker, add 3–5 g anhyd. Na_2SO_4, and stir gently to remove traces of H_2O. Decant ether ext. into another clean 400–500 ml beaker, and rinse Na_2SO_4 thoroly (ca 6 times) with small portions of ether. Combine rinses with ext.

(d) *Preparation of soln for chromatography.*—Evap. ext. on steam bath to vol. of ca 25 ml. Transfer to 50 ml beaker and continue evapn on steam bath until viscous oily residue forms which, when stirred with small rod, shows no indication of volatilizing liquid. Heat ca 20 sec., but not >2 min., until droplets of oil form. Remove from steam bath and immediately apply stream of *nitrogen* 1 min. Add 5 ml petr. ether, transfer to 10 ml vol. flask, and dil. to vol. with petr. ether. This is sample soln.

(e) *Alumina chromatography.*—Prep. chromatographic column (in 12 mm$\times$9 cm tube), by packing each adsorbent by gravity and slight tapping of tube. Add stdzd Al_2O_3 to height of 1 cm, then segment of alk. Al_2O_3 2 cm high, and another segment of stdzd Al_2O_3 4 cm high. Apply 5″ of vac., and add 5 ml petr. ether, followed by 5 ml of sample soln, then another 5 ml petr. ether. As last of soln disappears into column, add 5 ml portions of 16% ether eluent until all of carotene elutes from column. Det. completeness of elution by collecting final part of eluate in 1 ml beakers and observing color against white background. Elution is complete when carotene color cannot be seen in last 1 ml fraction observed. Combine all carotene fractions.

Continue elution with 5 ml portions of 16% ether. Examine column regularly with ultraviolet lamp and observe progress of fluorescent vitamin A band. (Total time required to elute vitamin A should be not >20 min. If it is desirable to accelerate movement of vitamin A down column, use 25% ether eluting solvent in 3 ml portions.) Discard eluate that collects after carotene fraction has been collected and before vitamin A band begins to elute. Collect all of vitamin A eluate in sep. beaker. Elution of vitamin A is complete when 1 ml portion of eluate collected in 1 ml beaker shows no vitamin A fluorescence when examined with ultraviolet lamp. Detn of cut-off point for collection of vitamin A fraction is very important, for if it goes too far, extraneous material absorbing at 325 mμ will be present, giving erroneously high results, and if chosen too early vitamin A values will be low. Combine all vitamin A-contg fractions. (NOTE: Some food dyes may not be sepd from vitamin A by Al_2O_3 chromatography. Whenever the fluorescent vitamin A eluate is colored, rechromatograph on MgO column as in (f).

Treat carotene eluate and vitamin A eluate separately, maintaining identity of each soln.

Reduce vol. of carotene eluate and of vitamin A eluate to ca 2 ml by evapn on steam bath. Remove remaining solvent completely by evapn at temp. not $>40°$ under vac. or with stream of N. Dissolve carotene in 5 ml of petr. ether, transfer to 10 ml vol. flask, and dil. to vol. with petr. ether. This is carotene soln for spectrophotometric measurement.

If vitamin A eluate shows no indication of color, dissolve residue in 5 ml absolute alcohol, transfer to 10 ml vol. flask, and dil. to vol. with absolute alcohol. If vitamin A eluate was colored, dissolve residue in ca 2 ml of petr. ether and proceed with chromatography on MgO-Celite.

(f) *Magnesium oxide chromatography.*—Add MgO-Celite mixt. to 12 mm$\times$6.5 cm tube, apply full vac., and tamp lightly. Column should be 4 cm high. Add 5 ml petr. ether and apply 15″ of vac. When petr. ether disappears into column, add petr. ether sample soln. Rinse container with three 2 ml portions petr. ether and add each rinse to column. Elute vitamin A from column with 0.5% absolute alcohol in petr. ether. Use technic of adding eluent, observing movement of vitamin A fluorescence, and collecting vitamin A eluate similar to that described for Al_2O_3 chromatography. This sepn should take not >10 min. Loss of vitamin A may result if this chromatographic step is too slow. Evap. solvent as before, dissolve residue in 5 ml absolute alcohol, transfer to 10 ml vol. flask, and dil. to vol. with absolute alcohol.

(g) *Spectrophotometric measurements and calculations.*—(1) *Carotene.*—Det. absorbance, A, of petr. ether soln of carotene at 450 mμ in 1 cm cell. Calc. mmg carotene/lb of sample, or of carotene as units of vitamin A/lb of sample, using formulae:

$$\text{mmg carotene/lb} = A \times 4.17 \times 455/W;$$

carotene as units vitamin A/lb

$$= A \times 6.95 \times 455/W;$$

where W = g sample/ml soln.

(2) *Vitamin A.*—Det. absorbance of absolute alcohol soln of vitamin A at 310 mμ and at 325 mμ in 1 cm cell. Calc. mmg or units of vitamin A/lb, using formulae:

$$\text{mmg vitamin A/lb} = A_{325} \times 5.5 \times 455/W;$$

$$\text{units vitamin A/lb} = A_{325} \times 18.3 \times 455/W;$$

where A_{325} = absorbance at 325 mμ and W = g sample/ml soln.

Det. ratio of absorbances at 310 to 325 mμ; this ratio is usually 1 or less.

Vitamin A in Mixed Feeds (*2*)—Official

(Work in subdued light. Avoid high laboratory temps. Complete all steps of method as rapidly as consistent with careful following of directions.)

39.008 APPARATUS

(a) *Photoelectric colorimeter.*—Evelyn or similar colorimeter or spectrophotometer with direct-reading deflection type galvanometer. Optical mechanism or filters to transmit light at 620 mμ. (Instrument which provides for linearity between absorbance and concn is preferable but not essential.) Use matched absorption tubes. For carotene detn, use 440 mμ filter or wavelength setting.

(b) *Carr-Price reagent dispenser.*—9 or 10 ml, delivering vol. of reagent rapidly thru 3–4 mm diam. opening. Use all glass app. Automatic pipet, hypodermic syringe, glass cylinder, or other types of app. may be used. App. must be clean and moisture-free.

(c) *Chromatographic tubes.*—18×200 mm (ca 12 mm i.d.), sealed to tube 5×100 mm.

(d) *Eluate receiver.*—Corning, special equipment, fraction collector No. 91200, or equiv.; or simple receiver consisting of 100 ml lipless graduated cylinder or 100 ml regular graduated cylinder with top cut off below lip and fitted with 2 hole stopper. Pass stem of chromatographic tube thru 1 hole and bent glass tube connected to source of vac. thru other. Ordinary H_2O aspirator may be used for vac.

(e) *Saponification apparatus.*—Any suitable ₸ joint, H_2O cooled, refluxing app. with 300–500 ml flask. Use boiling H_2O or steam for heat; or use special saponification and extn app. (Corning, special app., XA-4108, total capacity ca 500 ml).

(f) *Extraction apparatus.*—As above, or 500 ml separator, stopcock 4 mm, with device to push feed thru stopcock, if needed. To make device, use 2 hole stopper; thru 1 hole insert bent glass tube attached to rubber tube with glass mouthpiece. Thru other hole insert Fe or stainless steel wire with metal bead attached at ca midpoint so that when wire is raised bead will plug second hole and permit application of pressure; when wire is lowered, it will pass thru opened stopcock bore (*3*).

(g) *Ultraviolet light.*—Long wavelength, such as Mineralight model SL 5660 (Fisher Scientific Co., Cat. No. 11-984-1).

(h) *Evaporation assembly.*—To conc. exts after extn, if needed. Use H_2O aspirator and 60–65° H_2O bath (do not expose evapg soln and dry residue to atmosphere). Suitable assembly consists of Corning flask 4100 or 4320 of suitable size, connecting tube 8820 (₸ 19/38–24/40), and male ₸ 19/38 joint sealed to single arm of 2 mm bore, 3-way stopcock. One arm of stopcock is bent at 135° angle and attached to source of vac. thru trap; other arm is sealed to small reservoir made from test tube for adding solvent to dry residue as vac. is broken (*3*). For samples of high vitamin A potency, aliquot may be evapd directly in colorimetric tubes attached to source of vac. thru stopper and Y-tube. Optionally, evapn may be under partial vac. and N.

39.009 REAGENTS

(a) *Adsorbent.*—Use Woelm Alumina, non-alk. (Alupharm Chemicals, Box 755, 322–28 Lafayette, New Orleans, La.). Add 5% H_2O by placing measured quantity of H_2O in small g-s. bottle and distributing over walls; then add alumina and mix by shaking bottle until no lumps are observed. Let stand and cool at least 2 hr before use. Store in tightly closed bottle. (Woelm Alumina is heat-treated by manufacturer. Do not expose original or prepd alumina to air, since moisture content must be controlled carefully.)

(b) *Chloroform.*—Reagent grade. Purify if necessary by distn, discarding first and last 10%.

(c) *Hexane.*—Skellysolve B or other good quality commercial hexane. Redistill from all-glass app., using only 64–68° fraction. Solvent must be free from alcohols, esters, etc.

(d) *Alcohol.*—95%. Aldehyde free by Schiff's test.

(e) *Acetone in hexane soln.*—4% and 15%. Dil. reagent grade acetone with hexane.

(f) *Solution for removing antimony trichloride from tubes.*—Wash tubes with HCl, or let stand in 10% Rochelle salt soln to which detergent is added. Wash thoroly in hot detergent soln.

(g) *Potassium hydroxide soln.*—Dissolve 50 g

reagent grade KOH in H_2O and dil. to 100 ml with H_2O. Mix thoroly.

(h) *Antimony trichloride (Carr-Price) reagent.*—To 200 g $SbCl_3$ crystals add enough $CHCl_3$ to make 1 L. Warm and shake to dissolve. Cool, and add 30 ml Ac_2O. If soln is not clear, filter, centrifuge, or let settle and decant. Soln will keep in tightly stoppered brown bottle for several months. (Use fresh unopened bottle of reagent grade $SbCl_3$ crystals. Crystals should be translucent; no fluids or colored decomposition products should be present. Crystals stored too long or in previously opened bottle often fail to meet conditions. $SbCl_3$ contained in $\frac{1}{4}$ or $\frac{1}{2}$ lb g-s. bottles, with stopper sealed in, is recommended.)

(i) *Vitamin A reference solution.*—Soln of cryst. vitamin A acetate in cottonseed oil, encapsulated in gelatin. Potency 30 mg vitamin A/g oil or as stated at time of purchase. (30 mg vitamin A = 100,000 units.)

(j) *Carotene reference crystals.*—10% α-90% β carotene in sealed 100 mg or 200 mg vials, obtainable from General Biochemicals, 677 Laboratory Park, Chagrin Falls, Ohio. Crystals should dissolve in hexane without residue and have characteristic spectrophotometric curve. Det. concn, using spectrophotometer, as in **39.017.**

39.010 PREPARATION OF ADSORPTION COLUMN

Place small pledget of cotton at bottom of chromatographic tube and pack with adsorbent mixt. added in several portions, tamping each lightly with blunt rod, to depth of 7 cm. Keep column under vac. during packing. Add 0.5 cm layer of powd. anhyd. Na_2SO_4 on top of column, level, and pack lightly.

39.011 COLUMN PERFORMANCE TEST

Check for recovery of vitamin A as follows: Saponify ca 0.1 g USP vitamin A reference soln plus 2 g *fresh* cottonseed oil. Ext. with hexane. Mix soln of ca 50–100 mmg carotene and 30 mmg of the saponified vitamin A. Dil. to vol. of 15 ml. Wash column with 20 ml hexane and adjust elution rate to ca 2 drops/sec. Before top of column runs dry, add vitamin A-carotene mixt. Elute carotene with 4% acetone in hexane. (Approx. 20–30 ml will be required.) Check for vitamin A band by brief inspection with ultraviolet light. Band should be not >2 cm below top of alumina column.

Elute vitamin A with 15% acetone in hexane. (Approx. 30 ml should be enough.) Inspect last few ml of this eluate for vitamin A fluorescence, and if found, elute with few more ml of solvent, until fluorescence no longer is observed in eluate. Evap. suitable aliquot of eluate to dryness under vac., add 1 ml $CHCl_3$, and det. vitamin A. Compare result with aliquot of saponified vitamin A in hexane not chromatographed.

39.012 PREPARATION OF SAMPLE

Store sample in tightly closed glass containers at 0° or below. Grind to pass No. 20 sieve immediately before analyses. Mix and sample carefully.

39.013 DETERMINATION

Det. blank on all reagents, including cottonseed oil. Absorbance of this blank should be almost 0.

Preparation of std vitamin A curve.—Cut tip from capsule of Std Reference Soln and express appropriate quantity of oil into small weighed beaker or watch glass. Weigh accurately. Transfer oil to vol. flask and dil. to vol. with reagent grade $CHCl_3$. Use soln as soon as possible, but never after 8 hr. Work in subdued light or use low-actinic glassware. Make series of dilns of vitamin A soln (5 or more) with $CHCl_3$ so that 1 ml aliquots treated as for sample give transmissions of 20–85%. Plot absorbance against mmg vitamin A. On most photometers plot will be straight line in this transmission range and factor may be calcd for detg mmg vitamin A (*See Colorimetry*).

Preparation of std carotene curve.—Prep. series of dilns of α–β carotene in hexane. Plot absorbance against mmg carotene as in **39.017.** Use curve or factor for detg carotene content.

Determination of correction factor for yellow pigment in vitamin A eluate.—Cryptoxanthin and similar pigments may elute with vitamin A alcohol. Correct for this pigment if present in more than mere traces. Suitable correction may be obtained by saponification and extn of sample of yellow corn as for sample. Chromatograph and save 15% acetone in hexane fraction. Det. concn of yellow pigment in this fraction by comparison to carotene calibration. Evap. solvents and dissolve residue in $CHCl_3$. Make series of dilns covering range of concns of yellow pigment of sample solns in the 1 ml $CHCl_3$ on which Carr-Price vitamin A color is read. Obtain factor for correcting for reaction of this pigment in Carr-Price detn of vitamin A, by plotting concn of yellow pigment in soln detd at 440μ against concn of vitamin A detd from std vitamin A curve.

Saponification and extraction.—(1) For products <4000 units/lb, weigh 40 g sample into 500 ml boiling flask; (2) for products of 4000–20,000 units/lb, use 20–40 g samples; (3) for products of >20,000 units/lb, use 10 g sample. When analyzing premixes or concentrates of low fat content, add 1 g *fresh* cottonseed oil to sample in boiling flask.

Add vol. (ml) alcohol 3 times wt (g) sample. *Swirl until all particles are thoroly wetted.* Add vol.

KOH soln, **39.009(g)**, equal to wt sample. Swirl again for thoro mixing. Reflux 30 min. at rate of 2 drops/sec. Swirl and shake flasks until all clumps are broken and particles are well dispersed at least 3 times during digestion. Repeat once prior to extraction.

Cool to room temp. under running H_2O. Add vol. of H_2O 2 times wt of sample. Ext. 3 times with hexane, first time using vol. of hexane 2–3 times wt of sample, and ca $\frac{2}{3}$ as much for subsequent extns. On high potency samples it may be necessary to use larger vol. hexane or addnl extns. (If considerable quantities of carotenoid pigments are present, all are not removed in 3 extns, but those remaining in alc.-alk. layer are largely polyhydroxy and oxidized carotenoids.)

Combine all hexane exts in one separator. Pour 100 ml cool H_2O into separator and drain it when layers sep., retaining any emulsion in hexane layer. Repeat washing with successive 100 ml portions H_2O, with shaking, until washing is colorless to phthln. If emulsions cause difficulty, use 10% alcohol-H_2O wash contg 0.1% HCl on third washing. At least 2 H_2O washings should follow alcohol-H_2O wash. Sep. final H_2O wash as completely as possible. Swirl separator, let soln stand 5 min., and drain any H_2O collecting in bottom. Pour soln carefully from top of separator thru small pledget of cotton into appropriate vol. flask. Rinse separator and cotton with small portions of hexane, and dil. to vol. with hexane.

Chromatography.—If possible, chromatograph aliquot contg ca 30 mmg vitamin A, but preferably not <20, in 10–15 ml hexane ext. If necessary, conc. portion of hexane ext. under vac. to obtain enough concn of vitamin A. In no case should >25 ml soln be chromatographed.

Pack column as directed, wash with 20 ml hexane, and add ext. contg vitamin A just before top of column runs dry. Elute at rate of 2 drops /sec. Elute carotene and then vitamin A separately as above. Cryptoxanthin and similar pigments elute with vitamin A. Dil. carotene and also vitamin A eluate to vol. for colorimetry. Vol. of 50 ml of vitamin A eluate is convenient; 10 ml aliquot may be evapd for vitamin A detn.

Colorimetry.—Det. concn of carotene in carotene eluate as in **39.017.**

Transfer 10 ml vitamin A eluate to colorimeter tube and read yellow color as carotene. If this is more than trace, especially on low-potency samples, det. correction for yellow pigment as directed above.

Evap. solvents under vac. in hot (60–65°) H_2O bath. Dissolve residue in 1 ml $CHCl_3$. With 620 mμ setting, adjust colorimeter to 100% transmission, using 1 ml $CHCl_3$ and measured vol. (9 or 10 ml) $SbCl_3$ reagent. Place tube contg 1 ml $CHCl_3$ soln in instrument and add $SbCl_3$ reagent rapidly. Take max. reading of galvanometer (work rapidly; color begins to fade in 3–5 sec.). Examine tube within few sec. Soln should be blue and without turbidity; color should fade rapidly. Calc. wt vitamin A by reference to std vitamin A curve. Det. recovery factor, R, for vitamin A in analysis by addns of known quantities of vitamin A to duplicate samples or blank feeds in each series of similar samples analyzed.

Calc. vitamin A content of sample from formula:

Vitamin A (mmg/lb) $= (C_u - C_k) \times 454/C_s \times R$, where C_u = uncorrected concn of vitamin A in mmg/ml of final diln; C_k = correction for yellow pigment expressed as mmg vitamin A/ml of final diln; C_s = wt sample in g/ml of final diln; and R = vitamin A recovery factor.

Carotenes

Chromatographic Method (4)–Official

39.014 REAGENTS

(a) *Acetone.*—Dry, alcohol-free.

(b) *Commercial hexane.*—B.p. 60–70°.

(c) *Adsorbent.*—Activated magnesia (Micron brand, No. 2642; Westvaco Chlorine Products Co., Newark, Calif.) or SeaSorb 43.

(d) *Diatomaceous earth.*—Hyflo Super-Cel; Johns-Manville.

39.015 EXTRACTION

(a) *Hays and dried plants.*—Grind sample to pass No. 40 sieve. Weigh accurately 2 g sample (1 g if carotene content is high, 4 g if low) and place in flask of extractor (Goldfisch, Bailey-Walker, or ASTM is suitable if no thimble is used). Add 30 ml acetone-commercial hexane mixt. (3+7) to flask and (1) reflux 1 hr at rate of 1–3 drops/sec., and cool to room temp; or (2) stopper and place in dark at room temp. overnight (at least 15 hr). Decant or filter ext. into 100 ml vol. flask, wash residue, and dil. soln to vol. with hexane. (This soln now contains 9% acetone.)

(b) *Fresh plant materials and silages.*—Cut material finely with scissors or knife, or grind in food chopper to assure representative sample. If analysis cannot be performed immediately, blanch in boiling H_2O 5–10 min. and store in frozen condition. Place weighed sample, 2–5 g, in high speed blender; add 40 ml acetone, 60 ml hexane, and 0.1 g $MgCO_3$, and blend 5 min. Filter with suction or let residue settle and decant into separator, wash residue with two 25 ml portions acetone, then with 25 ml hexane, and combine exts. Wash acetone from ext. with five 100 ml portions H_2O, transfer upper layer to 100 ml vol.

flask contg 9 ml acetone, and dil. to vol. with hexane. If desired, alcohol may be used instead of acetone for extn. Use 80 ml alcohol and 60 ml hexane in blender; other quantities same as for acetone.

39.016 SEPARATION OF PIGMENTS

Prep. chromatographic column with 1+1 mixt. activated magnesia and diatomaceous earth. (Suitable chromatographic tube can be made from Pyrex test tube 22 mm o.d. and 175 mm long by sealing smaller tube (ca 10 mm o.d.) to bottom.) To prep. column, place small glass wool or cotton plug inside tube, add loose adsorbent to 15 cm depth, attach tube to suction flask, and apply full vac. of H_2O pump. Use flat instrument (such as inverted cork mounted on rod) to gently press adsorbent and flatten surface (packed column should be ca 10 cm deep). Place 1 cm layer anhyd. Na_2SO_4 above adsorbent.

With vac. continuously applied to flask, pour ext. into column. Use 50 ml acetone-hexane (1+9), or slightly more, if necessary, to develop chromatogram and wash visible carotenes thru adsorbent. Keep top of column covered with layer of solvent during entire operation (conveniently done by clamping inverted vol. flask full of solvent above column with neck 1–2 cm above surface of adsorbent).

Collect entire eluate. (Carotenes pass rapidly thru column; bands of xanthophylls, carotene oxidation products, and chlorophylls should be present in column when operation is complete.) Transfer eluate, which has been reduced in vol. by loss of vapor thru H_2O pump, to 100 ml vol. flask, and dil. to vol. with acetone-hexane (1+9). Det. carotene content of this soln photometrically.

39.017 DETERMINATION

Det. absorbance of soln as soon as possible with spectrophotometer at 436 mμ or with some other instrument provided with suitable filter system, such as Klett photometer with No. 44 filter, or Evelyn photoelec. colorimeter with 440 filter. Calibrate these instruments first with solns of high purity β-carotene as shown by characteristic absorption curve (*J. Biol. Chem.* **144**, 21(1942)). Prep. calibration chart and convert absorbance of soln to be detd to carotene concn from chart.

When detns are made with properly calibrated spectrophotometer at 436 mμ, calc. from formula

$$C = \frac{\text{Absorbance} \times 454}{196 \times L \times W}, \text{ where}$$

C = concn carotene (mg/lb) in original sample, L = cell length in cm, and W = g sample/ml final diln. Report results as mg β-carotene/lb. Multiply by 2.2 to give ppm or by 1667 to give International Units/lb.

Thiamine (Vitamin B_1)

(Methods not applicable in presence of materials that adsorb thiamine or which contain extraneous materials which affect thiochrome fluorescence.)

Fluorometric Method (5)—Official

39.018 REAGENTS AND APPARATUS

(a) *Double-normal sodium acetate.*—Dissolve 275 g $NaOAc.3H_2O$ in enough H_2O to make 1 L.

(b) *Bromocresol green pH indicator.*—Dissolve 0.1 g indicator by triturating in agate mortar with 2.8 ml 0.05N NaOH, and dil. to 200 ml with H_2O.

(c) *Thymol blue pH indicator.*—Dissolve 0.1 g indicator by triturating in agate mortar with 4.3 ml 0.05N NaOH, and dil. to 200 ml with H_2O.

(d) *Enzyme soln.*—Prep., on day on which it is to be used, 10% aq. soln of enzyme prepn potent in diastatic and phosphorolytic activity. (Among enzymes available for this purpose are Polidase-S (Schwarz Laboratories, 230 Washington St., Mount Vernon, N. Y.), Mylase P (Wallerstein Laboratories, 125 Lake Ave., Staten Island, N. Y.), Clarase (Takamine Laboratories, Clifton, N. J.), and Takadiastase (Parke, Davis & Co., Joseph Campau Ave. at the River, Detroit, Mich.)

(e) *Base-exchange silicate.*—Purify artificially prepd silicate of base-exchange type, in form of granular powder of "50–80 mesh" size, as follows: Place convenient quantity (100–500 g) base-exchange silicate in suitable beaker, add enough hot 3% HOAc to cover material, and boil 10–15 min., stirring continuously. Let mixt. settle and decant supernatant. Repeat washing 3 times, then wash similarly 3 times with hot KCl soln (1 part by wt KCl/4 vols soln), and finally wash with boiling H_2O (*distilled* H_2O must be used) until last washing gives no reaction for Cl. Dry material at ca 100° and store in well-closed container. (Purified base-exchange silicate may be purchased as "Special Decalso for Thiochrome Determination" from Fisher Scientific Co., 717 Forbes St., Pittsburgh 19, Pa.)

(f) *Chromatographic tubes.*—Use glass chromatographic tubes (ca 275 mm overall length, with reservoir capacity ca 60 ml) consisting of 3 parts fused together with following approx. i.d.: (1) reservoir at top, 95 mm long, 30 mm diam., converging into (2) adsorption tube, 145 mm long, 6 mm diam., and at lower end (3) tube is drawn into capillary 35 mm long and of such diam. that when tube is charged, rate of flow will be not

>1 ml/min. Prep. tubes for use as follows: Place over upper end of capillary, with aid of glass rod, pledget of fine glass wool. Add to adsorption tube H_2O suspension of 1.0–2.0 g purified base-exchange silicate, taking care to wash down all silicate from walls of reservoir. To keep air out of adsorption column, keep layer of liquid above surface of silicate during adsorption process. (Prevent tube from draining by placing rubber cap, filled with H_2O to avoid inclusion of air, over lower end of capillary.)

(**g**) *Neutral potassium chloride soln.*—Dissolve 250 g KCl in H_2O to make 1 L.

(**h**) *Acid potassium chloride soln.*—Add 8.5 ml HCl to 1 L of the neutral KCl soln.

(**i**) *Sodium hydroxide soln.*—15%. Dissolve 15 g NaOH in H_2O to make 100 ml.

(**j**) *Potassium ferricyanide soln.*—1%. Dissolve 1 g $K_3Fe(CN)_6$ in H_2O to make 100 ml. Prep. soln on day it is used.

(**k**) *Oxidizing reagent.*—Mix 4.0 ml of the 1% $K_3Fe(CN)_6$ soln with the 15% NaOH soln to make 100 ml. Use soln within 4 hr.

(**l**) *Isobutyl alcohol.*—Redistd in all-glass app. Use redistd product as anhyd. or H_2O-satd.

(**m**) *Quinine sulfate stock soln.*—Use quinine sulfate soln to govern reproducibility of fluorometer. Prep. stock soln of this reagent by dissolving 10 mg quinine sulfate in 0.1N H_2SO_4 to make 1 L. Store in light-resistant containers.

(**n**) *Quinine sulfate std soln.*—Dil. 1 vol. of the quinine sulfate stock soln with 39 vols 0.1N H_2SO_4. (Soln fluoresces to ca same degree as does isobutyl alcohol ext. of thiochrome obtained from 1 mmg thiamine.HCl.) Store soln in light-resistant containers.

(**o**) *Thiamine hydrochloride stock soln I.*—Weigh accurately 50–60 mg USP Thiamine Hydrochloride Reference Standard that has been dried to constant wt over P_2O_5 in desiccator. Since reference std is hygroscopic, take precautions to avoid absorption of moisture. Dissolve in 20% alcohol adjusted to pH 3.5–4.3 with HCl, and dil. to 500 ml with the acidified alcohol. Add enough addnl acidified alcohol to make concn exactly 100 mmg/ml. Store at ca 10° in g-s., light-resistant bottle.

(**p**) *Thiamine hydrochloride stock soln II.*—Dil. 100 ml thiamine.HCl stock soln I to 1 L with 20% alcohol adjusted to pH 3.5–4.3 with HCl. Store at ca 10° in g-s., light-resistant bottle. 1 ml = 10 mmg thiamine.HCl.

(**q**) *Thiamine hydrochloride std soln.*—To 10 ml thiamine.HCl stock soln II, add ca 50 ml ca 0.1N HCl, digest or autoclave as in **39.019(a)**(1), cool, and dil. to 100 ml with the 0.1N acid (1 ml equiv. to 1 mmg thiamine.HCl). Prep. fresh soln for each assay.

Proceed as in **39.020** and **39.021**, when assaying materials contg thiamine pyrophosphate, or as in **39.021** for materials contg free thiamine, using 20 ml aliquot of this soln. Designate final 25 ml vol. eluate (equiv. to 5 mmg USP Thiamine Hydrochloride Reference Standard) so obtained as Std Soln.

39.019 EXTRACTION

(**a**) *For materials that contain free thiamine (not applicable in presence of thiamine pyrophosphate).*—Place measured quantity of sample in flask of suitable size and prep. sample by (1), (2), or (3), and proceed directly to oxidation, **39.022.**

(1) *For dry or semidry materials that contain no appreciable quantity of basic substances.*—Add vol. 0.1N HCl equal in ml to not <10 times dry wt sample in g. Comminute and evenly disperse material in liquid if it is not readily sol. If lumping occurs, agitate vigorously so that all particles come in contact with liquid; then wash down sides of flask with 0.1N HCl. Digest 30 min. at 95–100° in steam bath, or in boiling H_2O, with frequent mixing; or autoclave mixt. 30 min. at 121–123°. Cool, and if lumping occurs, agitate mixt. until particles are evenly dispersed. Dil. with 0.1N HCl to measured vol. contg ca 0.2–5.0 mmg thiamine/ml.

(2) *For dry or semidry materials that contain appreciable quantities of basic substances.*—Add dil. HCl to adjust mixt. to ca pH 4.0. Add such quantity of H_2O that total vol. liquid is equal in ml to not <10 times dry wt sample in g. Add equiv. of 1 ml 10N HCl/100 ml liquid and proceed as in (1), beginning with second sentence.

(3) *For liquid materials.*—Adjust material to ca pH 4.0 with dil. HCl, or, with vigorous agitation, NaOH soln, and proceed as in (2), beginning with second sentence.

(**b**) *For materials that contain thiamine pyrophosphate.*—Proceed as in (**a**)(1), followed by enzyme hydrolysis and purification, **39.020–39.021.**

39.020 ENZYME HYDROLYSIS

Take aliquot contg ca 10–25 mmg thiamine, dil. to ca 65 ml with 0.1N HCl, and adjust pH to 4.0–4.5 with ca 5 ml of the 2N NaOAc, using bromocresol green indicator on spot plate. Add 5 ml of the enzyme soln, mix, and incubate 3 hr at 45–50°. Cool, dil. to 100 ml with 0.1N HCl, and filter thru paper known not to adsorb thiamine (ashfree papers have been found satisfactory).

39.021 PURIFICATION

Pass thru prepd chromatographic tube aliquot of the filtered soln contg ca 5 mmg thiamine, and wash column with three 5 ml portions of almost boiling H_2O, taking care to prevent surface of

liquid from falling below surface of the base-exchange silicate.

Elute thiamine from the base-exchange silicate by passing thru column five 4.0–4.5 ml portions of almost boiling acid-KCl soln, taking care to prevent surface of liquid from falling below surface of the silicate until final portion of acid-KCl soln has been added. Collect eluate in 25 ml vol. flask, cool, and dil. to vol. with the acid-KCl soln. Designate this as Assay Soln.

39.022 OXIDATION OF THIAMINE TO THIOCHROME

To each of 4 or more ca 40 ml tubes (or reaction vessels) add ca 1.5 g NaCl or KCl and 5 ml of the Std Soln. (*Precision and accuracy of results depend upon uniform technic in conducting following oxidation procedure.* Protect soln from light which destroys thiochrome. Use pipet that delivers 3 ml in 1–2 sec. for addn of the oxidizing reagent.) Place tip of pipet contg oxidizing reagent in neck of tube and hold it so that stream of soln does not hit side of tube. Swirl tube gently to produce rotary motion in liquid and immediately add 3 ml of the oxidizing reagent. Remove pipet and swirl tube again to insure adequate mixing. *Immediately thereafter,* add 13 ml isobutyl alcohol, stopper, and shake tube vigorously at least 15 sec. Treat 1 or more of the tubes similarly and treat each of 2 or more of remaining tubes (std blanks) in same manner except to replace the oxidizing reagent with the 15% NaOH soln.

To each of 4 or more similar tubes add 5 ml of the Assay Soln. Treat these tubes in same manner as directed for tubes contg the Std Soln.

After isobutyl alcohol has been added to all tubes, shake again ca 2 min. (Tubes may be placed in shaker box for this addnl shaking.) Centrifuge tubes at low speed until clear supernatant ext. can be obtained from each. Pipet or decant ca 10 ml of the isobutyl alcohol ext. (upper layer) from each tube into cell for measurement of thiochrome fluorescence.

39.023 THIOCHROME FLUORESCENCE MEASUREMENT

(Thiamine content of oxidized assay soln is detd by comparing intensity of fluorescence of ext. of this soln with that from oxidized std soln. Intensity of fluorescence is proportional to quantity thiamine present and may be measured with suitable electronic fluorometer. Input filter of narrow transmittance range with max. ca 365 mμ and output filter of narrow transmittance range with max. ca 435 mμ have been found satisfactory. Use quinine sulfate std soln to govern reproducibility of fluorometer.)

Measure fluorescence of isobutyl alcohol ext. from oxidized Assay Soln and call this reading A. Next measure fluorescence of the ext. from Assay Soln which has been treated with 3 ml of the 15% NaOH soln and call this reading (assay blank) b. Then measure fluorescence of the ext. from oxidized Std Soln and call this reading S. Finally, measure fluorescence of the ext. from Std Soln which has been treated with 3 ml of the 15% NaOH soln and call this reading (std blank) d.

39.024 CALCULATION

Calc. as follows:

Mmg thiamine hydrochloride in 5 ml Assay Soln $= (A - b)/(S - d)$.

Rapid Fluorometric Method (6)—Official

(Applicable to detn of thiamine in enriched flour, farina, corn meal, macaroni, and noodle products, or where bound thiamine or thiamine pyrophosphate is not significant.)

39.025 REAGENTS

See **39.018(c)**, **(i)**, **(j)**, **(k)**, **(l)**, **(m)**, **(n)**, **(o)**, and **(p)**.

39.026 PREPARATION OF STANDARD SOLUTION

Dil. 5 ml thiamine hydrochloride stock soln II, **39.018(p)**, to 250 ml with ca 0.1N HCl (1 ml = 0.2 mmg thiamine.HCl). Designate this as the Std Soln. If NaCl is to be added to sample for extn, add NaCl to Std Soln, before final diln, to give final concn of ca 5% w/v. Proceed as in **39.028.**

39.027 EXTRACTION

Weigh enough sample to give final assay soln with thiamine concn of ca 0.2 mmg/ml (*i.e.*, 4.54 g enriched flour for 100 ml or 9.07 g for 200 ml final vol.) and proceed by one of following methods:

(a) *95–100° digestion.*—Place measured quantity of sample in bottle or flask of suitable size. (Addn of NaCl to give final concn of ca 5% w/v aids in subsequent sepn of the sample soln. Mix flour and salt thoroly with stirring rod before adding the 0.1N HCl.) Add in 2 portions with vigorous stirring, vol. ca 0.1N HCl in proportion ca 15 ml acid to 1 g sample, using part of the acid to wash down sides of vessel. Place vessel in H_2O bath previously heated to 95–100°. Stir at frequent intervals to keep solids in suspension during thickening stage (5–8 min.) and occasionally during balance of total heating time of 30 min.

After hydrolysis has proceeded ca 10 min., place drop of soln on spot plate and test with thymol blue. Soln should be distinctly red (pH 1.0–1.2). If not acid enough (indicating presence of basic substances in sample), add ca 1N HCl in 1.0 ml amounts until desired acidity is reached. Note amount of 1N acid required to supplement the 0.1N acid and *repeat digestion* with new sample wt and necessary mixt. of 1N and 0.1N acids.

Cool, and dil. with $0.1N$ HCl to measured vol. that contains ca 0.2 mmg thiamine/ml.

Centrifuge mixt. until supernatant is clear or practically so and/or filter thru paper known not to adsorb thiamine (ash-free papers have been found satisfactory), or filter thru fritted glass funnel, using suitable analytical filter-aid (ash-free filter paper pulp and Celite Analytical Filter-Aid have been found satisfactory). Discard first $\frac{1}{10}$ part of filtrate. Designate remainder of filtrate as Assay Soln.

(**b**) *Autoclaved digestion.*—Proceed as in (**a**) without addn of NaCl, except to autoclave 20 min. at 5 lb pressure (108–109°) with total heating time not >35 min. including 5–10 min. to attain desired pressure and ca 5 min. to reduce pressure. (It may be necessary to preheat autoclave to ca 100° before inserting samples.)

39.028 OXIDATION

Proceed as in **39.022,** except add ca 2.5 g NaCl or KCl to each tube (or reaction vessel) before addn of the 5 ml Std Soln, **39.026,** or 5 ml Assay Soln, **39.027.** After addn of Std or Assay Soln, swirl each tube gently until most of salt is dissolved. Measure fluorescence of the isobutyl alcohol exts as in **39.023,** and calc. thiamine.HCl content as in **39.024.**

Thiamine in Bread (*7*)—Official

39.029 REAGENTS AND APPARATUS

Use reagents and app. as in **39.018** and following:

(**a**) *Thiamine hydrochloride stock soln III.*—Pipet 20 ml thiamine.HCl stock soln II, **39.018**(**p**), into 200 ml vol. flask and dil. to vol. with ca $0.1N$ HCl or $0.1N$ H_2SO_4 (1 ml = 1 mmg thiamine .HCl). Prep. fresh daily.

(**b**) *Procedural std.*—Pipet 40 ml stock soln III into one of the acid digestion containers, dil. to ca 150 ml with ca $0.1N$ HCl, and continue as under sample treatment (1 ml = 0.2 mmg thiamine .HCl in final vol.). (To be used for recovery experiments to test efficiency of method.)

(**c**) *Direct std.*—Pipet 40 ml stock soln III into 200 ml vol. flask, add ca 16 ml H_2O, and dil. to vol. with eluting reagent, **39.018**(**h**) (1 ml = 0.2 mmg thiamine.HCl).

39.030 ACID AND ENZYME DIGESTIONS

Weigh (±0.05 g) amount of air-dried bread, **13.090,** contg ca 40 mmg thiamine and transfer to 250 ml digestion flask or centrifuge bottle. Add 150 ml ca $0.1N$ HCl, stirring with glass rod to provide homogeneous mixt. with ca ½ of the acid and using remainder to wash down side of container. Digest 30 min. in boiling H_2O bath. Stir enough to prevent lumping or clotting, especially during first 5–10 min. Cool to room temp. and adjust pH to 4.5 by adding $2N$ NaOAc, **39.018**(**a**), with pH meter control or with bromocresol green indicator, **39.018**(**b**), and spot plate; end point should be definitely on blue side of the green-blue change.

Alternatively, use constant amount of hydrolyzing acid and previously detd amount of NaOAc soln required. Add 5 ml Polidase-S enzyme soln, **39.018**(**d**), mix, warm to 45°, and digest in H_2O bath 1 hr at 45–50°. Stir at 10–15 min. intervals. Cool, transfer, and dil. to vol. with H_2O in 200 ml vol. flask. Mix, and filter thru paper known not to adsorb thiamine. (Paper can be tested by comparing filtered and non-filtered procedural std, **39.029**(**b**). Ash-free papers have been found satisfactory.) Check pH of filtrate (should be at least 4.5 for subsequent base exchange sepn) and purify as in **39.021.**

39.031 OXIDATION OF THIAMINE TO THIOCHROME

Pipet duplicate 5 ml aliquots of Direct Std Soln, **39.029**(**c**), and duplicate 5 ml aliquots of Assay Soln into ca 40 ml tubes or reaction vessels, and proceed as in **39.022.**

39.032 THIOCHROME FLUORESCENCE MEASUREMENT

Thiamine content of oxidized Assay Soln is detd by comparing intensity of fluorescence of ext. of this soln with that from oxidized direct std soln, **39.029**(**c**), correcting for blank fluorescence of each of these solns. Intensity of fluorescence is linear in range 0–2 mmg thiamine and may be measured with suitable electronic fluorometer, **39.023.**

Mg thiamine.HCl/lb (fresh basis)

$$= \frac{A}{B} \times \frac{40 \times 454 \times F}{W \times 1000}.$$

if specified aliquots have been used, where A = corrected reading of assay soln; B = corrected reading of std soln; W = sample wt in g of air-dried bread; and F = air-dry wt: fresh wt ratio.

Riboflavin (Vitamin B_2) (*8*)

Fluorometric Method—Official

(Not applicable in presence of materials that adsorb riboflavin)

39.033 APPARATUS

Electronic photofluorometer.—Use fluorometer suitable for accurately measuring fluorescence of solns contg riboflavin in concns of 0.05–0.2 mmg/ml. Input filter of narrow transmittance range with max. ca 440 mμ and output filter of narrow transmittance range with max. ca 565 mμ have been found satisfactory.

39.034 REAGENTS

(a) *Riboflavin stock soln I.*—Dissolve 50 mg USP Riboflavin Reference Standard previously dried and stored in dark in desiccator over P_2O_5, in 0.02*N* HOAc to make 500 ml. (To facilitate soln, 50 mg Reference Standard may be added to ca 300 ml 0.02*N* HOAc and mixt. warmed on steam bath with constant stirring until riboflavin is dissolved, then cooled and 0.02*N* HOAc added to make 500 ml.) Store under toluene at ca 10°. 1.0 ml = 100 mmg riboflavin.

(b) *Riboflavin stock soln II.*—To 100 ml (a), add 0.02*N* HOAc soln to make 1 L. Store under toluene at ca 10°. 1.0 ml = 10 mmg riboflavin.

(c) *Riboflavin std soln.*—Dil. 10 ml (b) with H_2O to make 100 ml. 1 ml = 1 mmg riboflavin. Prep. fresh soln for each assay.

(d) *Sodium hydrosulfite.*—Powd. *See* **39.036**, NOTE.

39.035 PREPARATION OF SAMPLE SOLUTION

(Thruout procedure, keep solution <pH 7.0 to prevent loss of riboflavin)

Place measured quantity of sample in suitable size flask and proceed by one of following methods:

(a) *For dry or semidry materials that contain no appreciable quantity of basic substances.*—Add vol. 0.1*N* HCl equal in ml to not <10 times dry wt sample in g; resulting soln must not contain >0.1 mg riboflavin/ml. If material is not readily sol., comminute so that it may be evenly dispersed in liquid. Then agitate vigorously and wash down sides of flask with 0.1*N* HCl.

Heat mixt. in autoclave at 121–123° (1.1–1.2 kg/sq. cm) 30 min. and cool. If lumping occurs, agitate mixt. until particles are evenly dispersed. Adjust, with vigorous agitation, to pH 6.0–6.5 with NaOH soln; then add dil. HCl immediately until no further pptn occurs (usually ca pH 4.5, isoelec. point of many proteins).

Dil. mixt. to measured vol. contg >0.1 mmg riboflavin/ml and filter thru paper known not to adsorb riboflavin (ash-free papers have been found satisfactory). (In case of mixt. difficult to filter, centrifuging and/or filtering thru fritted glass, using suitable analytical filter-aid, may often be substituted for, or may precede, filtering thru paper. Ash-free filter paper pulp and Celite Analytical Filter-Aid have been found satisfactory.) Take aliquot of clear filtrate and check for dissolved protein by adding dropwise, first dil. HCl, and if no ppt forms, then, with vigorous agitation, NaOH soln, and proceed as follows:

(1) If no further pptn occurs, add, with vigorous agitation, NaOH soln to pH 6.8, dil. soln to final measured vol. contg ca 0.1 mmg riboflavin/ml, and if cloudiness occurs, filter again.

(2) If further pptn occurs, adjust soln again to point of max. pptn, dil. to measured vol. contg >0.1 mmg riboflavin/ml, and then filter. Take aliquot of clear filtrate and proceed as in (1).

If riboflavin content of sample is so low that these requirements cannot be met, conc. clear filtrate obtained at ca pH 4.5 to suitable vol. with heat under reduced pressure. Filter if necessary and proceed as in (1).

(b) *For dry or semidry materials that contain appreciable quantities of basic substances.*—Adjust mixt. to pH 5.0–6.0 with dil. HCl. Add such quantity of H_2O that total vol. liquid is equal in ml to not <10 times dry wt sample in g. (Resulting soln must not contain >0.1 mg riboflavin/ml.) Then add equiv. of 1.0 ml 10*N* HCl/100 ml liquid and proceed as in (a), beginning with second sentence.

(c) *For liquid materials.*—Adjust pH to 5.0–6.0 with dil. HCl or, with vigorous agitation, NaOH soln, and proceed as in (b), beginning with second sentence.

39.036 DETERMINATION

To each of 4 or more tubes (or reaction vessels) add 10 ml sample soln. (If fluorometer is type that requires tubular cuvettes, all reactions may be carried out in matched set of these cuvettes.) To each of 2 or more of these tubes add 1 ml of the std riboflavin soln and mix, and to each of 2 or more of remaining tubes, add 1 ml H_2O and mix. To each tube add 1 ml HOAc and mix; add, with mixing, 0.5 ml 4.0% $KMnO_4$ soln (quantity may be increased for sample solns that contain excess of oxidizable material, but not >0.5 ml in excess of that required to complete oxidation of foreign material should be added). Let stand 2 min.; then to each tube add, with mixing, 0.5 ml 3.0% H_2O_2 soln, whereupon permanganate color must be destroyed within 10 sec. Shake tubes vigorously until excess O is expelled. If gas bubbles remain on sides of tubes after foaming stops, remove by tipping tubes so that soln flows slowly from end to end.

In fluorometer, measure fluorescence of sample soln contg 1 ml added std riboflavin soln and call this reading "A." Next, measure fluorescence of the sample soln contg 1 ml added H_2O and call this reading "B." Add, with mixing, 20 mg *powd.* $Na_2S_2O_4$ to 2 or more tubes, measure fluorescence within 5 sec., and call reading "C." Calc. on basis of aliquots taken as follows:

Mg riboflavin/ml final sample soln $= [(B-C)/(A-B)] \times 0.10 \times 0.001$. (Value of $(B-C)/(A-B)$ must be not <0.66 nor >1.5.)

NOTE: The $Na_2S_2O_4$ must be of high purity and kept from undue exposure to light or air. Quantity

appreciably >20 mg may reduce foreign pigments and/or foreign fluorescing substances, thereby causing erroneous results. Suitability of the $Na_2S_2O_4$ may be checked as follows: To each of 2 or more tubes add 10 ml H_2O and 1 ml std riboflavin soln contg 20 mmg riboflavin/ml, and proceed as above with respect to addn of HOAc, $KMnO_4$ soln, and H_2O_2 soln. Then when 8 mg $Na_2S_2O_4$ is added, with mixing, riboflavin should be completely reduced in not >5 sec.

Nicotinic Acid (Niacin) and Nicotinamide (Niacinamide) (*9*)—Official

39.037 REAGENTS

(**a**) *Dilute ammonium hydroxide.*—Dil. 5 ml NH_4OH to 250 ml with H_2O.

(**b**) *Dilute hydrochloric acid.*—1+5.

(**c**) *Cyanogen bromide soln.*—10%. Prep. under hood. Warm 370 ml H_2O to 40° in large flask and add 40 g CNBr. Shake until dissolved, cool, and dil. to 400 ml. Do not let CNBr or soln come in contact with skin.

(**d**) *Sulfanilic acid soln.*—10%. Add NH_4OH in 1 ml portions to mixt. of 20 g sulfanilic acid and 170 ml H_2O until the acid dissolves. Adjust to pH 4.5 with HCl (1+1) with bromocresol green as outside indicator and dil. to 200 ml. Soln should be almost colorless.

(**e**) *Nicotinic acid stock soln.*—Dissolve 50 mg USP Nicotinic Acid Reference Standard, previously dried and stored in dark in desiccator over P_2O_5, in alcohol to make 500 ml. Store at ca 10°. 1 ml = 100 mmg nicotinic acid.

(**f**) *Nicotinic acid secondary std solns.*—Remove small portion nicotinic acid stock soln, (**e**), and let it come to room temp. Dil. 2 ml to 50 ml with H_2O for use in **39.038**(**c**) (1 ml = 4 mmg nicotinic acid).

39.038 DETERMINATION

(**a**) *Tablets or capsules.*—Disperse at least 5 tablets or capsules in small vol. H_2O with heat. Tablets may be ground first. Cool, transfer to vol. flask, and dil. to mark. Soln should contain 50–200 mmg nicotinic acid/ml. Pipet 10 ml aliquot into 250 ml erlenmeyer and add 10 ml HCl. Evap. on hot plate to ca 2 ml, cool, add ca 25–50 ml H_2O, and adjust to pH 7–9 by addn of few pellets NaOH or KOH. Filter, if necessary, and transfer to vol. flask of such size that soln after diln contains ca 5 mmg nicotinic acid/ml. Proceed as in (**c**).

(**b**) *Enriched foods and feeds.*—Weigh 1 oz sample (0.5 oz for glutenous materials) into 1 L erlenmeyer, add 200 ml 1*N* H_2SO_4, mix, and heat 30 min. in autoclave at 15 lb pressure. Cool, adjust to pH 4.5 with 10*N* NaOH, using bromocresol green as outside indicator, dil. to 250 ml with H_2O, and filter. (For materials contg bran, after autoclaving adjust to ca pH 13 by addn of 10*N* NaOH and let stand at room temp. 15 min. Then adjust to ca pH 4.5 by addn of HCl (3+1) and proceed as above.)

Proceed as in (**c**).

(**c**) Weigh 17 g $(NH_4)_2SO_4$ into 50 ml vol. flask, pipet in 40 ml aliquot of sample soln, dil. to mark with H_2O, and shake vigorously. Filter, mix well, and use 1 ml aliquot for color development. In case of samples with nicotinic acid content of 16 mg/lb, final soln contains 3.2 mmg/ml.

Pipet 40 ml aliquot std soln, **39.037**(**f**), into 17 g $(NH_4)_2SO_4$ in 50 ml vol. flask and dil. to mark with H_2O. This std contains 3.2 mmg/ml. Add sulfanilic acid and CNBr solns under hood from burets or from pipets filled by mechanical suction. *CNBr is toxic.* Prep. tubes as follows:

Standard Blank	*Sample Blank*
1.0 ml std soln	1.0 ml sample soln
5.0 ml H_2O	5.0 ml H_2O
0.5 ml dil. NH_4OH	0.5 ml dil. NH_4OH
2.0 ml sulfanilic acid	2.0 ml sulfanilic acid
0.5 ml dil. HCl	0.5 ml dil. HCl

Standard Soln	*Sample Soln*
1.0 ml std soln	1.0 ml sample soln
0.5 ml dil. NH_4OH	0.5 ml dil. NH_4OH
5.0 ml CNBr	5.0 ml CNBr
2.0 ml sulfanilic acid	2.0 ml sulfanilic acid
0.5 ml dil. HCl	0.5 ml dil. HCl

Prep. sep. sample blank for each sample.

Pipet std soln and sample soln into respective tubes (and in case of std blank or sample blank, add the H_2O). Add all subsequent solns to single tube and read color before proceeding with next tube. Swirl tube to impart rotary motion in liquid, add the dil. NH_4OH, immediately swirl again, add the sulfanilic acid, and swirl. Immediately add 0.5 ml dil. HCl, mix again, place in photoelec. colorimeter, and adjust instrument to 0 absorbance at any specific wavelength between 430 and 450 mμ within ca 30 sec. after addn of the sulfanilic acid soln. Treat std soln in same way as std blank with respect to addn of the dil. NH_4OH. Immediately swirl tube, add the CNBr soln, and swirl again. At 30 sec. after addn of the CNBr soln, swirl tube, add the sulfanilic acid soln, and swirl again. Immediately add 0.5 ml dil. HCl, mix again, and stopper. With instrument set at 0 absorbance for std soln blank as above, read absorbance of std soln at max. (Color reaches max, in ca 1.5 min. after addn of the sulfanilic acid soln, remains at peak ca 2 min., and then fades slowly.)

With sample blank set at 0 absorbance, det. absorbance of sample soln in same way. Nicotinic acid content is proportional to absorbance if std and sample solns are ca same concn.

Vitamin C (Ascorbic Acid) (*10*)—Official

(Applicable to orange, grapefruit, lemon, lime, and tomato juices provided they do not contain ferrous Fe, stannous Sn, cuprous Cu, SO_2, sulfite, or thiosulfate. *See* NOTE.)

39.039 REAGENTS

(a) *Metaphosphoric acid-acetic acid stabilizing extracting soln.*—Dissolve, with shaking, 15 g glacial HPO_3 pellets or freshly pulverized stick HPO_3 in 40 ml HOAc and 200 ml H_2O; dil. to ca 500 ml, and filter rapidly thru fluted paper into g-s. bottle. (HPO_3 slowly changes to H_3PO_4, but if stored in refrigerator, soln remains satisfactory 7–10 days.)

(b) *Ascorbic acid std.*—USP Reference L-Ascorbic Acid; keep cool, dry, and out of direct sunlight.

(c) *Indophenol std soln.*—Dissolve 50 mg 2,6-dichloroindophenol Na salt (Eastman No. 3463), that has been stored in desciccator over soda lime, in 50 ml H_2O to which has been added 42 mg $NaHCO_3$; shake vigorously, and when dye dissolves, dil. to 200 ml with H_2O. Filter thru fluted paper into amber g-s. bottle. Keep stoppered, out of direct sunlight, and store in refrigerator. (Decomposition products that make end point indistinct occur in some batches of dry indophenol and also develop with time in stock soln. Add 5.0 ml extg soln contg excess ascorbic acid to 15 ml dye reagent. If reduced soln is not practically colorless, discard, and prep. new stock soln. If dry dye is at fault, obtain new specimen.)

Weigh accurately (± 0.1 mg) ca 100 mg of the Reference Standard ascorbic acid, transfer to 100 ml g-s. vol. flask, and dil. to mark (room temp.) with the HPO_3-HOAc reagent. Stdze indophenol soln at once as follows: Transfer three 2.0 ml aliquots of the ascorbic acid soln to each of three 50 ml erlenmeyers contg 5.0 ml of the HPO_3-HOAc reagent. Titr. rapidly with the indophenol soln from 50 ml buret until light but distinct rose-pink persists at least 5 sec. (Each titrn should require ca 15 ml of the indophenol soln, and titrns should check within 0.1 ml.) Similarly titr. 3 blanks composed of 7.0 ml of the HPO_3-HOAc reagent plus vol. H_2O ca equiv. to vol. indophenol soln used in direct titrns. After subtracting av. blanks (usually ca 0.1 ml) from stdzn titrns, calc. and express concn of indophenol soln as mg ascorbic acid equiv. to 1.0 ml reagent. Stdze indophenol soln daily with freshly prepd std ascorbic acid soln.

39.040 PREPARATION OF SAMPLE AND DETERMINATION

Prep. juice as in **20.002(a)**. Add aliquots of at least 100 ml prepd juice to equal vols of the HPO_3-HOAc reagent. Mix, and filter rapidly thru rapid folded paper (Eaton-Dikeman No. 195, 18.5 cm, or equiv.). Titr. 10 ml aliquots, and make blank detns for corrections of titrns as in **39.039(c)**, using proper vols of acid reagent and H_2O. Express ascorbic acid as mg/100 ml original juice.

NOTE: Products contg ferrous Fe, stannous Sn, and cuprous Cu give values in excess of their actual ascorbic acid content by this method. Following are simple tests to det. whether these reducing ions are present in such quantities as to invalidate test: Add 2 drops *0.05% aq. soln of methylene blue* to 10 ml freshly prepd mixt. (1+1) of juice and the HPO_3-HOAc reagent and mix. Disappearance of methylene blue color in 5–10 sec. indicates presence of interfering substances. Stannous Sn does not give this test and may be tested for as follows: To another 10 ml sample soln to which 10 ml HCl (1+3) has been added, add 5 drops *0.05% aq. soln of indigo carmine* and mix. Disappearance of color in 5–10 sec. indicates presence of stannous Sn or other interfering substance.

MICROBIOLOGICAL METHODS

General

(Thruout all stages, except where otherwise directed, protect solns from undue exposure to light.)

39.041 STOCK SOLUTIONS FOR BASAL MEDIA

(Store all solns in dark at ca 10°. Proportionate quantities may be prepd.)

(a) *Acid-hydrolyzed casein soln.*—Mix 400 g vitamin-free casein with 2 L constant-boiling HCl (ca 20% HCl) and either reflux 8–12 hr, or heat in autoclave 8–12 hr at 121–123°. Remove HCl from mixt. by distn under reduced pressure until thick paste remains. Redissolve paste in H_2O, adjust soln to pH 3.5 ± 0.1 with ca 10% NaOH soln, and dil. with H_2O to 4 L. Add to soln 80 g activated charcoal, stir 1 hr, and filter. Repeat treatment with activated charcoal. Store under toluene. Filter soln if ppt forms upon storage. (Some commercial sources of vitamin-free acid-hydrolyzed casein have been found satisfactory.)

(b) *Adenine-guanine-uracil soln.*—Dissolve 0.7 g each of adenine sulfate, guanine.HCl and uracil in 35 ml warm HCl (1+1), cool, and dil. with H_2O to 700 ml. Store under toluene.

(c) *Asparagine soln.*—Dissolve 8 g L-asparagine.H_2O in H_2O and dil. to 800 ml. Store under toluene.

(d) *Cystine soln.*—Suspend 2 g L-cystine in ca 750 ml H_2O, heat to 70–80°, and add HCl (1+1), dropwise, with stirring, until solid dissolves. Cool, and dil. with H_2O to 1 L. Store under toluene.

(e) *Cystine-tryptophan soln.*—Suspend 8 g L-cystine and 2 g L-tryptophan (or 4 g D,L-tryptophan) in ca 1.5 L H_2O, heat to 70–80°, and add HCl (1+1), dropwise, with stirring, until solids

dissolve. Cool, and dil. with H_2O to 2 L. Store under toluene.

(f) *Manganese sulfate soln.*—Dissolve 2 g $MnSO_4.H_2O$ in H_2O and dil. to 200 ml. Store under toluene.

(g) *Photolyzed peptone soln.*—Dissolve 100 g peptone in 625 ml H_2O, add soln of 50 g NaOH in 625 ml H_2O, and mix in vessel (such as crystg dish) of such size that depth of soln is 1–2 cm. Place 100–500 watt bulb, fitted with reflector, ca 30–50 cm from soln, and expose soln, with occasional stirring, to light from bulb until riboflavin is destroyed (4–10 hr may be enough). Maintain soln at not >25° during this treatment. Adjust soln to pH 6.0–6.5 with HOAc, add 18 g anhyd. NaOAc, stir until solid dissolves, dil. with H_2O to 2 L, and filter if soln is not clear. Store under toluene.

(h) *Polysorbate 80 soln.*—Dissolve 25 g polysorbate 80 (polyoxyethylene sorbitan monooleate) in alcohol to make 250 ml.

(i) *Salt soln A.*—Dissolve 40 g anhyd. KH_2PO_4 and 40 g anhyd. K_2HPO_4 in H_2O and dil. to 800 ml. Add 8 drops HCl and store under toluene.

(j) *Salt soln B.*—Dissolve 20 g $MgSO_4.7H_2O$, 1 g NaCl, 1 g $FeSO_4.7H_2O$, and 1 g $MnSO_4.H_2O$ in H_2O and dil. to 1 L. Add 10 drops HCl and store under toluene.

(k) *Tryptophan soln.*—Suspend 2.0 g L-tryptophan (or 4.0 g D,L-tryptophan) in 700–800 ml H_2O, heat to 70–80°, and add HCl (1+1), dropwise, with stirring, until solid dissolves. Cool, and dil. with H_2O to 1 L. Store under toluene.

(l) *Vitamin soln I.*—Dissolve 10 mg riboflavin, 10 mg thiamine.HCl, 0.1 mg biotin, and 20 mg nicotinic acid in $0.02N$ HOAc to make 400 ml. Store under toluene.

(m) *Vitamin soln II.*—Dissolve 20 mg *p*-aminobenzoic acid, 10 mg Ca pantothenate, 40 mg pyridoxine.HCl, 40 mg pyridoxal.HCl, 8 mg pyridoxamine.2HCl, and 2 mg folic acid in 25% alcohol to make 400 ml.

(n) *Vitamin soln III.*—Dissolve 10 mg *p*-

39.042 BASAL MEDIA STOCK SOLUTIONS FOR 250 ML (PROPORTIONATE QUANTITIES MAY BE PREPD)[a]

Ingredients (Stock Solutions, 39.041)	(a) Cobalamin (Vitamin B_{12} Activity)	(b) Folic Acid (Pteroylglutamic Acid)	(c) Nicotinic Acid and Nicotinamide (Niacin and Niacinamide)	(d) Pantothenic Acid	(e) Riboflavin (Vitamin B_2)
	ml	ml	ml	ml	ml
(a) Acid-hydrolyzed casein soln	25	25	25	25	
(b) Adenine-guanine-uracil soln	5	2.5	5	5	
(c) Asparagine soln	5	15			
(d) Cystine soln					25
(e) Cystine-tryptophan soln			25	25	
(f) Manganese sulfate soln		5			
(g) Photolyzed peptone soln					50
(h) Polysorbate 80 soln	5	0.25		0.25	
(i) Salt soln A	5		5	5	5
(j) Salt soln B	5	5	5	5	5
(k) Tryptophan soln		25			
(l) Vitamin soln I	10				
(m) Vitamin soln II	10				
(n) Vitamin soln III		50			
(o) Vitamin soln IV			5	5	
(p) Vitamin soln V			5		
(q) Vitamin soln VI				5	
(r) Xanthine soln	5	5			
(s) Yeast supplement soln					5
Solids	grams	grams	grams	grams	grams
Ascorbic acid	1				
L-Cysteine.HCl.H_2O		0.19			
L-Cystine	0.1				
Dextrose, anhyd.	10	10	10	10	15
Glutathione		0.0013			
K_2HPO_4, anhyd.		1.6			
NaOAc, anhyd.	5		5	5	
Na citrate.2H_2O		13			
D,L-Tryptophan	0.1				

[a] Some commercial sources of basal media have been found satisfactory.

aminobenzoic acid, 40 mg pyridoxine.HCl, 4 mg thiamine.HCl, 8 mg Ca pantothenate, 8 mg nicotinic acid, and 0.2 mg biotin in ca 300 ml H_2O. Add 10 mg riboflavin dissolved in ca 200 ml 0.02*N* HOAc. Then add soln contg 1.9 g anhyd. NaOAc and 1.6 ml HOAc in ca 40 ml H_2O, and dil. with H_2O to 2 L. Store under toluene.

(**o**) *Vitamin soln IV.*—Dissolve 8 mg riboflavin, 4 mg thiamine.HCl, and 0.016 mg biotin in 0.02*N* HOAc to make 400 ml. Store under toluene.

(**p**) *Vitamin soln V.*—Dissolve 2 mg *p*-aminobenzoic acid, 4 mg Ca pantothenate, and 8 mg pyridoxine.HCl in 25% alcohol to make 200 ml.

(**q**) *Vitamin soln VI.*—Dissolve 2 mg *p*-aminobenzoic acid, 10 mg nicotinic acid, and 8 mg pyridoxine.HCl in 25% alcohol to make 200 ml.

(**r**) *Xanthine soln.*—Suspend 0.4 g xanthine in 60–80 ml H_2O, heat to ca 70°, add 12 ml NH_4OH (2+3), and stir until solid dissolves. Cool, and dil. with H_2O to 400 ml. Store under toluene.

(**s**) *Yeast supplement soln.*—Dissolve 20 g H_2O-sol. yeast ext. in 100 ml H_2O, add soln of 30 g Pb subacetate in 100 ml H_2O (soln is turbid), and mix. Filter, and adjust filtrate to pH 10 with NH_4OH (1+2). Filter, and adjust filtrate to pH 6.5 with HOAc. Ppt excess Pb with H_2S, filter, and dil. filtrate with H_2O to 200 ml. Store under toluene.

39.043 CULTURE AND SUSPENSION MEDIA

(**a**) *Liquid culture medium.*—Dissolve 15 g peptonized milk, 5 g H_2O-sol. yeast ext., 10 g anhyd. dextrose, and 2 g anhyd. KH_2PO_4 in ca 600 ml H_2O. Add 100 ml filtered tomato juice, and adjust to pH 6.5–6.8 with NaOH soln. Add, with mixing, 10 ml polysorbate 80 soln, **39.041**(**h**), and dil. with H_2O to 1 L. Add 10 ml portions of the soln to test tubes, plug with cotton, sterilize 15 min. in autoclave at 121–123°, and cool tubes as rapidly as practicable to keep color formation at min. Store in dark at ca 10°. (Difco liquid culture medium for AOAC microbiological assays has been found satisfactory.)

(**b**) *Agar culture medium.*—To 500 ml liquid culture medium (**a**), add 5.0–7.5 g agar, and heat with stirring on steam bath until agar dissolves. Add ca 10 ml portions of the hot soln to test tubes, plug with cotton, sterilize 15 min. in autoclave at 121–123°, and cool tubes in upright position as rapidly as practicable to keep color formation at min. Store in dark at ca 10°. (Difco agar culture medium for AOAC microbiological assays (Lactobacilli Agar Loy) has been found satisfactory.)

(**c**) *Suspension medium.*—Dil. measured vol. appropriate basal medium stock soln, **39.042**, with equal vol. H_2O. Add 10 ml portions of dild medium to test tubes, plug with cotton, sterilize 15 min. in autoclave at 121–123°, and cool tubes as rapidly as practicable to keep color formation at min. Store in dark at ca 10°.

39.044 STOCK CULTURES OF TEST ORGANISMS

For appropriate test organism, designated below, prep. stab culture in 1 or more tubes of *agar culture medium*, **39.043**(**b**). Incubate 6–24 hr at any selected temp. between 30° and 40° held constant to within ±0.5°, and finally store in dark at ca 10°. Before using new culture in assay, make several successive transfers of the culture in 1–2 week period.

Prep. fresh stab culture 1 or more times weekly and do not use for prepg inoculum if >1 week old.

Activity of slow-growing culture may be increased by daily or twice-daily transfer of stab culture, and is considered satisfactory when definite turbidity in liquid inoculum can be observed 2–4 hr after inoculation. Slow-growing culture seldom gives suitable response curve and may cause erratic results.

(**a**) *Lactobacillus leichmanii.*—ATCC No. 7830. For use in assay of cobalamin.

(**b**) *Streptococcus faecalis.*—ATCC No. 8043. For use in assay of folic acid.

(**c**) *Lactobacillus plantarum.*—ATCC No. 8014. For use in assay of nicotinic acid and pantothenic acid.

(**d**) *Lactobacillus casei.*—ATCC No. 7469. For use in assay of riboflavin.

39.045 ASSAY TUBES

Cleanse meticulously by suitable means (Na lauryl sulfate USP has been found satisfactory as detergent), hard-glass test tubes, ca 20×150 mm, and other necessary glassware. (Test organisms are highly sensitive to minute amounts of growth factors and to many cleansing agents. Therefore, it may be preferred to follow cleansing by heating 1–2 hr at ca 250°. This is of particular importance in cobalamin assay.)

Prep. tubes contg appropriate std soln as follows: To test tubes add, in duplicate (or replicate), 0.0 (for uninoculated blanks), 0.0 (for inoculated blanks), 1.0, 2.0, 3.0, 4.0, and 5.0 ml, resp., of the std soln.

Prep. tubes contg appropriate assay soln as follows: To similar test tubes add, in duplicate (or replicate), 1.0, 2.0, 3.0, and 4.0 ml, resp., of the assay soln.

To each tube of std soln and assay soln add H_2O to make 5.0 ml. Then add 5.0 ml appropriate basal medium stock soln, **39.042**, and mix. Cover tubes suitably to prevent bacterial contamination, and sterilize (10 min. for titrimetric method,

39.046; or 5 min. for turbidimetric method, **39.048**) in autoclave at 121–123°, reaching this temp. in not >10 min. Cool as rapidly as practicable to keep color formation at min. Take precautions to keep sterilizing and cooling conditions uniform thruout assay. Too close packing of tubes in autoclave, or overloading of it, may cause variation in heating rate.

Aseptically inoculate each tube, except 1 set of duplicate (or replicate) tubes contg 0.0 ml std soln (uninoculated blanks), with 1 drop appropriate inoculum. Incubate for time period designated in titrimetric method, **39.046**, or turbidimetric method, **39.048**, at any selected temp. between 30° and 40° held constant to within ±0.5°. Contamination of assay tubes with any foreign organism invalidates assay.

Titrimetric Method

39.046 DETERMINATION

Incubate tubes 72 hr, and then titr. contents of each tube with 0.1*N* NaOH, using bromothymol blue indicator, or to pH 6.8 measured electrometrically.

Disregard results of assay if response at inoculated blank level is equiv. to titrn of >1.5 ml greater than that at uninoculated blank level. Response at 5.0 ml level of std soln should be equiv. to titrn of ca 8–12 ml.

Prep. std concn-response curve by plotting titrn values, expressed in ml 0.1*N* NaOH for each level of std soln used, against quantity of reference std contained in respective tubes.

Det. quantity of vitamin for each level of assay soln by interpolation from std curve. Discard any observed titrn values equiv. to <0.5 ml, or >4.5 ml, resp., of the std soln. Proceed as in **39.049**.

Turbidimetric Method

(Not applicable in the presence of extraneous turbidity or color in amount that interferes with turbidimetric measurements.)

39.047 CALIBRATION OF PHOTOMETER

Using inoculum and std stock soln as prescribed for appropriate vitamin in following table, and using suspension medium **39.043(c)**, proceed as directed below.

	Cobalamin	Folic Acid	Nicotinic Acid	Pantothenic Acid	Riboflavin
Inoculum	39.052[a]	39.060	39.072	39.081	39.090
Std Stock Soln	39.051(a)	39.059(b)	39.067(a)	39.076(a)	39.085(a)

[a] Proceed as in 39.052, except replace fifth sentence with the following: "Dil. 0.2–1.0 ml aliquot of this suspension with 10 ml of the sterile suspension medium."

Add aseptically 1 ml inoculum to ca 300 ml sterile suspension medium contg 1.0 ml std stock soln, and incubate mixt. for same period and at same temp. to be employed in detn, **39.048**. After incubating, centrifuge and wash cells 3 times with ca 50 ml portions 0.9% NaCl soln; then resuspend cells in the NaCl soln to make 25 ml.

Evap. 10 ml aliquot of cell suspension on steam bath, and dry to constant wt at 100° in vac. oven. Correcting for wt of NaCl, calc. dry wt of cells in mg/ml of suspension.

Dil. second measured aliquot of cell suspension with 0.9% NaCl soln so that each ml is equiv. to 0.5 mg dry cells. To test tubes add, in triplicate, 0.0 (for blanks), 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, and 5.0 ml, resp., of this dild cell suspension. To each tube add 0.9% NaCl soln to make 5.0 ml. Then add 5.0 ml appropriate basal medium stock soln, **39.042**, mix (1 drop of suitable *antifoam agent* may be added; 1–2% soln of Dow Corning Antifoam AF Emulsion or Antifoam B has been found satisfactory), and transfer to optical cell. With blanks set at 100% transmittance, measure % transmittance of contents of each tube under same conditions to be used in respective assay. Prep. curve by plotting % transmittance readings for each level of dild cell suspension used against cell content (mg dry wt) of respective tubes.

Repeat appropriate calibration step at least 2 more times for photometer to be used in respective assay. Draw composite curve, best representing 3 or more individual curves, relating % transmittance to mg dried cell wt for photometer under conditions of respective assay. Once appropriate curve for particular instrument is established, all subsequent relationships between % transmittance and cell wt are detd directly from this curve. Respective assay limits expressed as mg dried cell wt/tube are so detd.

39.048 DETERMINATION

Incubate tubes 16–24 hr until max. turbidity is obtained, as demonstrated by lack of significant change during 2 hr addnl incubation period in tubes contg highest level of std soln.

Det. transmittance of tubes as follows: Mix thoroly contents of each tube (1 drop of suitable antifoam agent soln may be added; 1–2% soln of Dow Corning Antifoam AF Emulsion or Antifoam B has been found satisfactory), and transfer to optical cell. Agitate contents, place cell in photometer set at any specific wavelength between 540 and 660 mμ, and read % transmittance when steady state is reached.

Steady state is observed few sec. after agitation

when galvanometer reading remains constant 30 sec. or more. Allow ca same time interval for reading on each tube.

With transmittance set at 100% for uninoculated blank level, read % transmittance of inoculated blank level. If this reading corresponds to dried cell wt >0.6 mg/tube, disregard results of assay. Then with transmittance reset at 100% for inoculated blank level, read % transmittance for each of remaining tubes. Disregard results of assay if % transmittance observed at 5.0 ml level of std soln (against inoculated blank) is equiv. to that for dried cell wt of <1.25 mg/tube.

Prep. std concn-response curve by plotting % transmittance readings for each level of std soln used, against quantity of reference std contained in respective tubes.

Det. quantity of vitamin for each level of assay soln by interpolation from std curve. Discard any observed transmittance values equiv. to <0.5 ml, or >4.5 ml, resp., of the std soln. Proceed as in **39.049**.

39.049 CALCULATION FOR BOTH TITRIMETRIC AND TURBIDIMETRIC METHODS

For each level of assay soln used, calc. vitamin content/ml of assay soln. Calc. av. of values obtained from tubes that do not vary by $> \pm 10\%$ from this av. If the number of acceptable values remaining is $<\frac{2}{3}$ of original number of tubes used in the 4 levels of assay soln, data are insufficient for calcg potency of sample. If number of acceptable values remaining is $\frac{2}{3}$ or more of original number of tubes, calc. potency of sample from av. of them.

Cobalamin (Vitamin B_{12} Activity) (*11*)

(Applicable to materials contg ca 0.1 mmg or more of Vitamin B_{12} activity/g or ml)

39.050 BASAL MEDIUM STOCK SOLUTION

Using ingredients in amounts prescribed for cobalamin, **39.042**(**a**), proceed as directed below.

Dissolve the L-cystine and D,L-tryptophan in 10 ml 1*N* HCl. Using solns prepd as in **39.041**, add, with mixing, and in following order: adenine-guanine-uracil soln, (**b**); xanthine soln, (**r**); vitamin soln I, (**l**); vitamin soln II, (**m**); salt soln A, (**i**); salt soln B, (**j**); asparagine soln, (**c**); and acid-hydrolyzed casein soln, (**a**). Add ca 100 ml H_2O and add, with mixing, the anhyd. dextrose, anhyd. NaOAc, and ascorbic acid. When soln is complete, adjust to pH 6.0 with NaOH soln, add, with mixing, polysorbate 80 soln, (**h**), and dil. with H_2O to 250 ml.

Titrimetric Method

39.051 CYANOCOBALAMIN STANDARD SOLUTIONS

(**a**) *Stock soln I*.—Weigh accurately, in closed system, USP Cyanocobalamin Reference Standard, equiv. to 50–60 mmg cyanocobalamin, that has been dried to constant wt and stored in dark over P_2O_5 in desiccator. Dissolve in 25% alcohol, and dil. with addnl 25% alcohol to make cyanocobalamin concn exactly 100 millimicrograms/ml. Store in dark at ca 10°.

(**b**) *Stock soln II*.—Dil. 10 ml stock soln I, (**a**), with 25% alcohol to 1 L. Store in dark at ca 10°. 1.0 ml = 1.0 millimicrogram cyanocobalamin.

(**c**) *Std soln*.—Dil. suitable quantity of stock soln II, (**b**), with H_2O to measured vol. such that after incubation as in **39.045** and **39.046**, response at the 5.0 ml level of this soln is equiv. to titrn (as described in **39.046**) of ca 8–12 ml. Designate this as std soln. (This concn is usually 0.01–0.04 millimicrogram cyanocobalamin/ml std soln.) Prep. fresh std soln for each assay.

39.052 INOCULUM

Make transfer of cells from stock culture of *Lactobacillus leichmannii*, **39.044**(**a**), to sterile tube contg 10 ml liquid culture medium, **39.043**(**a**). Incubate 6–24 hr at any selected temp. between 30 and 40° held constant to within ±0.5°. Under aseptic conditions, centrifuge culture and decant supernatant. Suspend cells from culture in 10 ml sterile suspension medium, **39.043**(**c**). Dil. aliquot with the sterile suspension medium to give transmittance equiv. to that for dried cell wt (as described in **39.047**) of 0.50–0.75 mg per tube when read against suspension medium set at 100% transmittance. Cell suspension so obtained is the inoculum.

39.053 ASSAY SOLUTION

Prep. aq. extg soln just before use contg, in each 100 ml, 1.3 g anhyd. Na_2HPO_4, 1.2 g citric acid monohydrate, and 1.0 g *anhyd. Na metabisulfite*, $Na_2S_2O_5$. Place measured quantity of sample in flask contg at least 25 ml extg soln for each g or ml sample taken. If sample is not readily sol., comminute so that it may be evenly dispersed in liquid; then agitate vigorously and wash down sides of flask with H_2O.

Autoclave mixt. 10 min. at 121–123° and cool. If lumping occurs, agitate mixt. until particles are evenly dispersed. Dil. mixt to measured vol. with H_2O, and let any undissolved particles settle, or filter or centrifuge if necessary. Take aliquot of clear soln, add H_2O, adjust to pH 6.0, and dil. with addnl H_2O to measured vol. contg, per ml, cobalamin activity ca equiv. to that of the std soln, **39.051**(**c**). Designate this as assay soln. Excess of bisulfite may affect test organism. Therefore, assay soln must contain not >0.03 mg $Na_2S_2O_5$/ml.

39.054 ASSAY

Using std soln, **39.051**(**c**), assay soln, **39.053**, basal medium stock soln, **39.050**, and inoculum, **39.052**, proceed as in **39.045**, **39.046**, and **39.049**.

Turbidimetric Method

39.055 CYANOCOBALAMIN STANDARD SOLUTION

Dil. suitable quantity of cyanocobalamin std stock soln II, **39.051(b)**, with H_2O to measured vol. such that after incubation as in **39.045** and **39.048**, with inoculated blank set at 100% transmittance, % transmittance at 5.0 ml level of this soln is equiv. to that for dried cell wt (as described in **39.047**) of not <1.25 mg. Designate this as std soln. (This concn is usually 0.01–0.04 millimicrogram cyanocobalamin/ml std soln.) Prep. fresh std soln for each assay.

39.056 ASSAY SOLUTION

Proceed as in **39.053** except that where reference is made to concn of cyanocobalamin activity ca equiv. to that of the std soln, **39.051(c)**, replace by concn of cyanocobalamin activity ca equiv. to that of the std soln, **39.055**. Designate soln so obtained as assay soln.

39.057 ASSAY

Using std soln, **39.055**, assay soln, **39.056**, basal medium stock soln, **39.050**, and inoculum, **39.052**, proceed as in **39.045**, **39.048**, and **39.049**.

Folic Acid (Pteroylglutamic Acid) (*12*)

(Applicable only to materials contg free forms of folic acid.)

39.058 BASAL MEDIUM STOCK SOLUTION

Using ingredients in amounts prescribed for folic acid, **39.042(b)**, proceed as directed below.

Using solns prepd as in **39.041** add, with mixing, and in following order: acid-hydrolyzed casein soln, (**a**); tryptophan soln, (**k**); adenine-guanine-uracil soln, (**b**); xanthine soln, (**r**); asparagine soln, (**c**); vitamin soln III, (**n**); and salt soln B, (**j**). Add ca 50 ml H_2O, and add, with mixing, the cysteine, anhyd. dextrose, Na citrate dihydrate, anhyd. K_2HPO_4, and glutathione. When soln is complete, adjust to pH 6.8 with NaOH soln, add, with mixing, polysorbate 80 soln, (**h**), and $MnSO_4$ soln, (**f**), and dil. with H_2O to 250 ml.

Titrimetric Method

39.059 FOLIC ACID STANDARD SOLUTIONS

(**a**) *Stock soln I.*—Weigh accurately, in closed system, USP Folic Acid Reference Standard, equiv. to 50–60 mg folic acid, that has been dried to constant wt and stored in dark over P_2O_5 in desiccator. Dissolve in ca 30 ml 0.01*N* NaOH, add ca 300 ml H_2O, adjust to pH 7–8 with HCl soln, and dil. with addnl H_2O to make folic acid concn exactly 100 mmg/ml. Store under toluene in dark at ca 10°.

(**b**) *Stock soln II.*—To 10 ml stock soln I, (**a**). add ca 500 ml H_2O, adjust to pH 7–8, and dil. with addnl H_2O to 1 L. Store under toluene in dark at ca 10°. 1.0 ml = 1.0 mmg folic acid.

(**c**) *Stock soln III.*—To 100 ml stock soln II, (**b**), add ca 500 ml H_2O, adjust to pH 7–8, and dil. with addnl H_2O to 1 L. Store under toluene in dark at ca 10°. 1.0 ml = 100 millimicrograms folic acid.

(**d**) *Std soln.*—Dil. suitable quantity of stock soln III, (**c**), with H_2O to measured vol. such that after incubation as in **39.045** and **39.046**, response at 5.0 ml level of this soln is equiv. to titrn (as described in **39.046**) of ca 8–12 ml. Designate this as std soln. (This concn is usually 1.0–4.0 millimicrograms folic acid/ml std soln.) Prep. fresh std soln for each assay.

39.060 INOCULUM

Make transfer of cells from stock culture of *Streptococcus faecalis*, **39.044(b)**, to sterile tube contg 10 ml liquid culture medium, **39.043(a)**. Incubate 6–24 hr at any selected temp. between 30° and 40° held constant to within ±0.5°. Under aseptic conditions, centrifuge culture and decant supernatant. Suspend cells from culture in 10 ml sterile suspension medium, **39.043(c)**. Cell suspension so obtained is the inoculum.

39.061 ASSAY SOLUTION

Place measured quantity of sample in flask and add vol. H_2O equal in ml to not <10 times dry wt sample in g; resulting soln must contain not >1.0 mg folic acid/ml. Add equiv. of 2 ml NH_4OH (2+3)/100 ml liquid. If sample is not readily sol., comminute so that it may be evenly dispersed in liquid; then agitate vigorously and wash down sides of flask with 0.1*N* NH_4OH.

Autoclave mixt. 15 min. at 121–123° and cool. If lumping occurs, agitate mixt. until particles are evenly dispersed. Dil. mixt. to measured vol. with H_2O, and let any undissolved particles settle, or filter or centrifuge if necessary. Take aliquot of the clear soln, add H_2O, adjust to pH 6.8, and dil. with addnl H_2O to measured vol. contg, per ml, folic acid ca equiv. to that of std soln, **39.059(d)**. Designate this as assay soln.

39.062 ASSAY

Using std soln, **39.059(d)**, assay soln, **39.061**, basal medium stock soln, **39.058**, and inoculum, **39.060**, proceed as in **39.045**, **39.046**, and **39.049**.

Turbidimetric Method

39.063 FOLIC ACID STANDARD SOLUTION

Dil. suitable quantity of folic acid std stock soln III, **39.059(c)**, with H_2O to measured vol. such that after incubation as in **39.045** and

39.048, with inoculated blank set at 100% transmittance, % transmittance at 5.0 ml level of this soln is equiv. to that for dried cell wt (as described in 39.047) of not <1.25 mg. Designate this as std soln. (This concn is usually 0.5–2.0 millimicrograms folic acid/ml std soln.) Prep. fresh std soln for each assay.

39.064 ASSAY SOLUTION

Proceed as in **39.061** except that where reference is made to folic acid concn ca equiv. to that of std soln, **39.059(d)**, replace by folic acid concn ca equiv. to that of std soln, **39.063.** Designate soln so obtained as assay soln.

39.065 ASSAY

Using std soln, **39.063,** assay soln, **39.064,** basal medium stock soln, **39.058,** and inoculum, **39.060,** proceed as in **39.045, 39.048,** and **39.049.**

Nicotinic Acid and Nicotinamide (Niacin and Niacinamide) (*13*)

39.066 BASAL MEDIUM STOCK SOLUTION

Using ingredients in amounts prescribed for nicotinic acid, **39.042(c),** proceed as directed below.

Using solns prepd as in **39.041,** add, with mixing, and in following order: acid-hydrolyzed casein soln, (**a**); cystine-tryptophan soln (**e**); adenine-guanine-uracil soln, (**b**); vitamin soln IV, (**o**); vitamin soln V, (**p**); salt soln A, (**i**); and salt soln B, (**j**). Add ca 100 ml H_2O, and add, with mixing, the anhyd. dextrose and anhyd. NaOAc. When soln is complete, adjust to pH 6.8 with NaOH soln, and dil. with H_2O to 250 ml.

Titrimetric Method

39.067 NICOTINIC ACID STANDARD SOLUTIONS

(**a**) *Stock soln I.*—Weigh accurately, in closed system, 50–60 mg USP Nicotinic Acid Reference Standard that has been dried to constant wt and stored in dark over P_2O_5 in desiccator. Dissolve in 25% alcohol, and dil. with addnl 25% alcohol to make nicotinic acid concn exactly 100 mmg/ml. Store in dark at ca 10°.

(**b**) *Stock soln II.*—Dil. 100 ml stock soln I, (**a**), with 25% alcohol to 1 L. Store in dark at ca 10°. 1.0 ml = 10 mmg nicotinic acid.

(**c**) *Std soln.*—Dil. suitable quantity of stock soln II, (**b**), with H_2O to measured vol. such that after incubation as in **39.045** and **39.046** response at 5.0 ml level of this soln is equiv. to titrn (as described in **39.046**) of ca 8–12 ml. Designate this as std soln. (This concn is usually 0.1–0.4 mmg nicotinic acid/ml std soln.) Prep. fresh std soln for each assay.

39.068 INOCULUM

(**a**) *Liquid culture medium.*—Dil. measured vol. basal medium stock soln, **39.066,** with equal vol. aq. soln contg 0.2 mmg nicotinic acid/ml. Add 10 ml portions of the dild medium to test tubes, plug with cotton, sterilize 15 min. in autoclave at 121–123°, and cool tubes as rapidly as practicable to avoid color formation from overheating. Store in dark at ca 10°.

(**b**) *Inoculum.*—Make transfer of cells from stock culture of *Lactobacillus plantarum,* **39.044(c),** to sterile tube contg 10 ml liquid culture medium, (**a**). Incubate 6–24 hr at any selected temp. between 30° and 40° held constant to within ±0.5°. Cell suspension so obtained is the inoculum.

39.069 ASSAY SOLUTION

Place measured quantity of sample in flask and proceed as directed below. Designate final measured vol. so obtained as assay soln.

(**a**) *For dry or semidry materials that contain no appreciable quantity of basic substances.*—Add vol. $1N$ H_2SO_4 equal in ml to not <10 times dry wt sample in g; resulting soln must contain not >5.0 mg nicotinic acid/ml. If sample is not readily sol., comminute so that it may be evenly dispersed in liquid. Then agitate vigorously and wash down sides of flask with $1N$ H_2SO_4.

Autoclave mixt. 30 min. at 121–123° and cool. If lumping occurs, agitate mixt. until particles are evenly dispersed. If dissolved protein is not present, adjust mixt. to pH 6.8 with NaOH soln, dil. with H_2O to final measured vol. contg, per ml, nicotinic acid ca equiv. to that of std soln, **39.067(c),** and filter if soln is not clear.

If dissolved protein is present, adjust mixt., with vigorous agitation, to pH 6.0–6.5 with NaOH soln; then add dil. HCl immediately until no further pptn occurs (usually ca pH 4.5, isoelectric point of many proteins). Dil. mixt. to measured vol. with H_2O, and filter. (In case of mixt. difficult to filter, centrifuging and/or filtering thru fritted glass, using suitable analytical filter-aid, may often be substituted for, or may precede, filtering thru paper. Ash-free filter paper pulp and Celite Analytical Filter-Aid have been found satisfactory.) Take aliquot of clear filtrate and check for dissolved protein by adding dropwise, first dil. HCl, and if no ppt forms, then, with vigorous agitation, NaOH soln, and proceed as follows with this aliquot:

(1) If no further pptn occurs, add, with vigorous agitation, NaOH soln to pH 6.8, dil. with H_2O to final measured vol. contg, per ml, nicotinic acid ca equiv. to that of std soln, **39.067(c),** and if cloudiness occurs, refilter.

(2) If further pptn occurs, adjust mixt. again to point of max. pptn, dil. with H_2O to measured

vol., and then filter. Take aliquot of clear filtrate and proceed as in (1).

(**b**) *For dry or semidry materials that contain appreciable quantities of basic substances.*—Adjust mixt. to pH 5.0–6.0 with dil. H_2SO_4. Add vol. H_2O equal in ml to not <10 times dry wt sample in g; resulting soln must contain not >5.0 mg nicotinic acid/ml. Then add equiv. of 10 ml 10*N* H_2SO_4/100 ml liquid and proceed as in (**a**), beginning with second sentence, "If sample is not readily sol., . . . "

(**c**) *For liquid materials.*—Adjust mixt. to pH 5.0–6.0 with H_2SO_4 soln or NaOH soln and proceed as in (**b**), beginning with second sentence, "Add vol. H_2O . . . "

39.070 ASSAY

Using std soln, **39.067**(**c**), assay soln, **39.069**, basal medium stock soln, **39.066**, and inoculum, **39.068**(**b**), proceed as in **39.045**, **39.046**, and **39.049**. Value so obtained is potency of sample expressed as nicotinic acid equiv. Multiply this value by 0.992 if potency is to be expressed as nicotinamide equiv.

Turbidimetric Method

39.071 NICOTINIC ACID STANDARD SOLUTIONS

(**a**) *Stock soln III.*—Dil. 10 ml nicotinic acid std stock soln I, **39.067**(**a**), with 25% alcohol to 1 L. Store in dark at ca 10°. 1.0 ml = 1.0 mmg nicotinic acid.

(**b**) *Std soln.*—Dil. suitable quantity of nicotinic acid stock soln III, (**a**), with H_2O to measured vol. such that after incubation as in **39.045** and **39.048**, with inoculated blank set at 100% transmittance, % transmittance at 5.0 ml level of this soln is equiv. to that for dried cell wt (as described in **39.047**) of not <1.25 mg. Designate this as std soln. (This concn is usually 0.01–0.04 mmg nicotinic acid/ml std soln.) Prep. fresh std soln for each assay.

39.072 INOCULUM

Proceed as in **39.068**(**b**). Then under aseptic conditions, centrifuge culture so obtained and decant supernatant. Suspend cells from culture in 10 ml sterile suspension medium, **39.043**(**c**). Cell suspension so obtained is the inoculum.

39.073 ASSAY SOLUTION

Proceed as in **39.069** except that where reference is made to nicotinic acid concn ca equiv. to that of std soln, **39.067**(**c**), replace by nicotinic acid concn ca equiv. to that of std soln, **39.071**(**b**). Designate soln so obtained as assay soln.

39.074 ASSAY

Using std soln, **39.071**(**b**), assay soln, **39.073**, basal medium stock soln, **39.066**, and inoculum, **39.072**, proceed as in **39.045**, **39.048**, and **39.049**. Value so obtained is potency of sample expressed as nicotinic acid equiv. Multiply this value by 0.992 if potency is to be expressed as nicotinamide equiv.

Pantothenic Acid (*14*)

(Applicable only to materials contg Ca pantothenate or other free forms of pantothenic acid.)

39.075 BASAL MEDIUM STOCK SOLUTION

Using ingredients in amounts prescribed for pantothenic acid, **39.042**(**d**), proceed as directed below.

Using solns prepd as in **39.041**, add, with mixing, and in following order: acid-hydrolyzed casein soln, (**a**); cystine-tryptophan soln, (**e**); adenine-guanine-uracil soln, (**b**); vitamin soln IV, (**o**); vitamin soln VI, (**q**); salt soln A, (**i**); and salt soln B, (**j**). Add ca 100 ml H_2O and add, with mixing, the anhyd. dextrose and anhyd. NaOAc. When soln is complete, adjust to pH 6.8 with NaOH soln, add, with mixing, polysorbate 80 soln, (**h**), and dil. with H_2O to 250 ml.

Titrimetric Method

39.076 PANTOTHENIC ACID STANDARD SOLUTIONS

(**a**) *Stock soln I.*—Weigh accurately, in closed system, 45–55 mg USP Calcium Pantothenate Reference Standard that has been dried to constant wt and stored in dark over P_2O_5 in desiccator. Dissolve in ca 500 ml H_2O, add 10 ml 0.2*N* HOAc and 100 ml 0.2*N* NaOAc, and dil. with addnl H_2O to make Ca pantothenate concn exactly 43.47 mmg/ml. Store under toluene in dark at ca 10°. 1.0 ml = 40 mmg pantothenic acid.

(**b**) *Stock soln II.*—To 25 ml stock soln I, (**a**), add ca 500 ml H_2O, 10 ml 0.2*N* HOAc, and 100 ml 0.2*N* NaOAc, and dil. with addnl H_2O to 1 L. Store under toluene in dark at ca 10°. 1.0 ml = 1.0 mmg pantothenic acid.

(**c**) *Std soln.*—Dil. suitable quantity of stock soln II, (**b**), with H_2O to measured vol. such that after incubation as in **39.045** and **39.046**, response at 5.0 ml level of this soln is equiv. to titrn (as described in **39.046**) of ca 8–12 ml. Designate this as std soln. (This concn is usually 0.005–0.020 mmg pantothenic acid/ml std soln.) Prep. fresh std soln for each assay.

39.077 INOCULUM

(**a**) *Liquid culture medium.*—Dil. measured vol. basal medium stock soln, **39.075**, with equal vol. aq. soln contg 0.04 mmg pantothenic acid/ml. Add 10 ml portions of the dild medium to test tubes,

plug with cotton, sterilize 15 min. in autoclave at 121–123°, and cool tubes as rapidly as practicable to avoid color formation from overheating. Store in dark at ca 10°.

(**b**) Make transfer of cells from stock culture of *Lactobacillus plantarum*, **39.044(c)**, to sterile tube contg 10 ml liquid culture medium, (**a**). Incubate 6–24 hr at any selected temp. between 30° and 40° held constant to within ±0.5°. Cell suspension so obtained is the inoculum.

39.078 ASSAY SOLUTION

(Thruout all stages of procedure, keep solution below pH 7.0 to prevent loss of pantothenic acid.)

Place measured quantity of sample in flask and proceed as directed below. (Where directed to filter thru paper, use paper known not to adsorb pantothenic acid. Ash-free papers have been found satisfactory.) Designate final measured vol. so obtained as assay soln.

(**a**) *For dry or semidry materials that contain no appreciable quantity of basic substances.*—Add vol. H_2O equal in ml to not <10 times dry wt sample in g; resulting soln must contain not >5 mg pantothenic acid/ml. Adjust mixt. to pH 5.65 ±0.05 with HOAc soln or NaOAc soln. If sample is not readily sol., comminute so that it may be evenly dispersed in liquid. Then agitate vigorously and wash down sides of flask with aq. soln contg in each liter 10 ml 0.2*N* HOAc and 100 ml 0.2*N* NaOAc.

Autoclave mixt. 5–7 min. at 121–123° and cool. Then proceed as in **39.069(a)**, beginning with second sentence in second paragraph, "If lumping occurs . . ." except where reference is made to nicotinic acid concn ca equiv. to that of std soln, **39.067(c)**, replace by pantothenic acid concn ca equiv. to that of std soln, **39.076(c)**.

(**b**) *For dry or semidry materials that contain appreciable quantities of basic substances.*—Adjust mixt. to pH 5.0–6.0 with HOAc soln. Add vol. H_2O equal in ml to not <10 times dry wt sample in g; resulting soln must contain not >5 mg pantothenic acid/ml. Then proceed as in (**a**) beginning with second sentence: "Adjust mixt. to pH 5.65 ±0.05 . . ."

(**c**) *For liquid materials.*—Adjust mixt. to pH 5.0–6.0 with HOAc soln or NaOAc soln and proceed as in (**b**), beginning with second sentence: "Add vol. H_2O . . ."

39.079 ASSAY

Using std soln, **39.076(c)**, assay soln, **39.078**, basal medium stock soln, **39.075**, and inoculum, **39.077(b)**, proceed as in **39.045**, **39.046**, and **39.049**. Value so obtained is potency of sample expressed as D-pantothenic acid equiv. Multiply this value by 1.087 if potency is to be expressed as Ca D-pantothenate equiv.

Turbidimetric Method

39.080 PANTOTHENIC ACID STANDARD SOLUTION

Dil. suitable quantity of pantothenic acid stock soln II, **39.076(b)**, with H_2O to measured vol. such that after incubation as in **39.045** and **39.048**, with inoculated blank set at 100% transmittance, % transmittance at 5.0 ml level of this soln is equiv. to that for dried cell wt (as described in **39.047**) of not <1.25 mg. Designate this as std soln. (This concn is usually 0.003–0.012 mmg pantothenic acid/ml std soln.) Prep. fresh std soln for each assay.

39.081 INOCULUM

Proceed as in **39.077(b)**. Then under aseptic conditions, centrifuge culture so obtained and decant supernatant. Suspend cells from culture in 10 ml sterile suspension medium, **39.043(c)**. Cell suspension so obtained is the inoculum.

39.082 ASSAY SOLUTION

Proceed as in **39.078** except that where reference is made to pantothenic acid concn ca equiv. to that of std soln, **39.076(c)**, replace by pantothenic acid concn ca equiv. to that of std soln, **39.080**. Designate soln so obtained as assay soln.

39.083 ASSAY

Using std soln, **39.080**, assay soln, **39.082**, basal medium stock soln, **39.075**, and inoculum, **39.081**, proceed as in **39.045**, **39.048**, and **39.049**. Value so obtained is potency of sample expressed as D-pantothenic acid equiv. Multiply this value by 1.087 if potency is to be expressed as Ca D-pantothenate equiv.

Riboflavin (Vitamin B_2) (*15*)

(Not applicable in presence of materials which absorb riboflavin.)

39.084 BASAL MEDIUM STOCK SOLUTION

Using ingredients in amounts prescribed for riboflavin, **39.042(e)**, proceed as directed below.

Using solns prepd as in **39.041**, add, with mixing, and in the following order: photolyzed peptone soln, (**g**); cystine soln, (**d**); yeast supplement soln, (**s**); salt soln A, (**i**); and salt soln B, (**j**). Add ca 100 ml H_2O, and add, with mixing, the anhyd. dextrose. When soln is complete, adjust to pH 6.8 with NaOH soln, and dil. with H_2O to 250 ml.

Titrimetric Method

39.085 RIBOFLAVIN STANDARD SOLUTIONS

(**a**) *Stock soln I.*—Weigh accurately, in closed system, 50–60 mg USP Riboflavin Reference Standard that has been dried to constant wt and stored in dark over P_2O_5 in desiccator. Suspend in

ca 300 ml 0.02*N* HOAc and warm on steam bath, with stirring, until solid dissolves. Cool, and dil. with 0.02*N* HOAc to make riboflavin concn exactly 100 mmg/ml. Store under toluene in dark at ca 10°.

(b) *Stock soln II.*—Dil. 100 ml stock soln I, (a), with 0.02*N* HOAc to 1 L. Store under toluene in dark at ca 10°. 1.0 ml = 10 mmg riboflavin.

(c) *Std soln.*—Dil. suitable quantity of stock soln II, (b), with H_2O to measured vol. such that after incubation as in **39.045** and **39.046,** response at 5.0 ml level of this soln is equiv. to titrn (as described in **39.046**) of ca 8–12 ml. Designate this as std soln. (This concn is usually 0.05–0.20 mmg riboflavin/ml std soln.) Prep. fresh std soln for each assay.

39.086 INOCULUM

(a) *Liquid culture medium.*—Dil. measured vol. basal medium stock soln, **39.084,** with equal vol. aq. soln contg 0.1 mmg riboflavin/ml. Add 10 ml portions of the dild medium to test tubes, plug with cotton, sterilize 15 min. in autoclave at 121–123°, and cool tubes as rapidly as practicable to avoid color formation from overheating. Store in dark at ca 10°.

(b) Make transfer of cells from stock culture of *Lactobacillus casei,* **39.044**(d), to sterile tube contg 10 ml liquid culture medium, (a). Incubate 6–24 hr at any selected temp. between 30° and 40° held constant to within ±0.5°. Cell suspension so obtained is inoculum.

39.087 ASSAY SOLUTION

(Thruout all stages of procedure, keep solution below pH 7.0 to prevent loss of riboflavin.)

Place measured quantity of sample in flask and proceed as directed below. (Where directed to filter thru paper, use paper known not to adsorb riboflavin. Ash-free papers have been found satisfactory.) Designate final measured vol. so obtained as assay soln.

(a) *For dry or semidry materials that contain no appreciable quantity of basic substances.*—Add vol. 0.1*N* HCl equal in ml to not <10 times dry wt sample in g; resulting soln must contain not >0.1 mg riboflavin/ml. If sample is not readily sol., comminute so that it may be evenly dispersed in liquid. Then agitate vigorously and wash down sides of flask with 0.1*N* HCl.

Then proceed as in **39.069**(a), beginning with second par., "Autoclave mixt. 30 min. . . . " except that where reference is made to nicotinic acid concn ca equiv. to that of std soln, **39.067**(c), replace by riboflavin concn ca equiv. to that of std soln, 39.085(c).

If riboflavin content of sample is so low that these requirements cannot be met, conc. clear filtrate obtained at ca pH 4.5 to suitable vol. with heat under reduced pressure. Filter if necessary, and proceed as in **39.069**(a)(1).

(b) *For dry or semidry materials that contain appreciable quantities of basic substances.*—Adjust mixt. to pH 5.0–6.0 with dil. HCl. Add vol. H_2O equal in ml to not <10 times dry wt sample in g; resulting soln must contain not >0.1 mg riboflavin/ml. Then add equiv. of 1.0 ml 10*N* HCl/100 ml liquid and proceed as in (a), beginning with second sentence, "If sample is not readily sol. . . . "

(c) *For liquid materials.*—Adjust mixt. to pH 5.0–6.0 with dil. HCl, or with vigorous agitation, NaOH soln, and proceed as in (b), beginning with second sentence, "Add vol. H_2O . . . "

39.088 ASSAY

Using std soln, **39.085**(c), assay soln, **39.087,** basal medium stock soln, **39.084,** and inoculum, **39.086**(b), proceed as in **39.045, 39.046,** and **39.049.**

Turbidimetric Method

39.089 RIBOFLAVIN STANDARD SOLUTIONS

(a) *Stock soln III.*—Dil. 10 ml riboflavin stock soln I, **39.085**(a), with 0.02*N* HOAc to 1 L. Store under toluene in dark at ca 10°. 1.0 ml = 1.0 mmg riboflavin.

(b) *Std soln.*—Dil. suitable quantity of stock soln III, (a), with H_2O to measured vol. such that after incubation as in **39.045** and **39.048,** with inoculated blank set at 100% transmittance, % transmittance at 5.0 ml level of this soln is equiv. to that for dried cell wt (as described in **39.047**) of not <1.25 mg. Designate this as std soln. (This concn is usually 0.01–0.04 mmg riboflavin/ml std soln.) Prep. fresh std soln for each assay.

39.090 INOCULUM

Proceed as in **39.086**(b). Then under aseptic conditions, centrifuge culture so obtained and decant supernatant. Suspend cells from culture in 10 ml sterile suspension medium, **39.043**(c). Cell suspension so obtained is the inoculum.

39.091 ASSAY SOLUTION

Proceed as in **39.087** except that where reference is made to riboflavin concn ca equiv. to that of std soln, **39.085**(c), replace by riboflavin concn ca equiv. to that of std soln, **39.089**(b). Designate soln so obtained as assay soln.

39.092 ASSAY

Using std soln, **39.089**(b), assay soln, **39.091,** basal medium stock soln, **39.084,** and inoculum, **39.090,** proceed as in **39.045, 39.048,** and **39.049.**

Amino Acids (*16*)—First Action

(Applicable only to materials contg free forms of amino acids in absence of appreciable quantity of protein.)

39.093 STOCK SOLUTIONS FOR BASAL MEDIA

(a) *Amino acid soln.*—Prep. stock soln of each amino acid as in table, **39.094.** Use amino acids of highest purity available. (DL form is preferred since it is less likely to be contaminated with other amino acids.) If DL form is used, double wt given in table. Dissolve amino acid with heat, if necessary, and dil. to indicated vol. with H_2O. Where special solvents are indicated, dissolve amino acid in vol. initial solvent specified, and dil. to final vol. with H_2O. Store solns under toluene in dark at ca 10°.

(b) *Adenine-guanine-uracil soln.*—*See* **39.041**(b).

(c) *Salt soln A.*—Dissolve 50 g anhyd. K_2HPO_4 and 50 g anhyd. KH_2PO_4 in H_2O and dil. to 500 ml. Add 5 drops HCl and store under toluene.

(d) *Salt soln B.*—Dissolve 20 g $MgSO_4.7H_2O$, 1 g NaCl, 1 g $FeSO_4.7H_2O$, and 1 g $MnSO_4.H_2O$ in H_2O, and dil. to 500 ml. Add 5 drops HCl and store under toluene.

(e) *Vitamin soln I.*—Dissolve 25 mg riboflavin, 25 mg thiamine.HCl, 50 mg nicotinic acid, and 0.15 mg biotin in 0.02*N* HOAc to make 1 L. Store under toluene in dark at ca 10°.

(f) *Vitamin soln II.*—Dissolve 50 mg pyridoxal, 50 mg pyridoxamine.HCl, 25 mg Ca pantothenate, 5 mg *p*-aminobenzoic acid, and 0.25 mg folic acid in 25% alcohol to make 1 L. Store in dark at ca 10°.

39.095 BASAL MEDIUM

Complete assay medium is given in table, **39.096.** In prepg medium for given assay, omit particular amino acid to be assayed from medium. Concn of all ingredients in basal medium is twice that it will be in assay tube after addn of sample or std soln and H_2O. Table lists amounts of ingredients required for 50, 100, 150, and 200 tubes. Prep. fresh for each assay.

To combined solns add the dextrose and NaOAc with mixing. When soln is complete, adjust to pH 6.8 and dil. to vol. (NOTE: Quantities of dextrose and NaOAc depend upon organism used in assay.)

(*See next page for* **39.096**)

39.097 CULTURE AND SUSPENSION MEDIA

(a) *Agar culture medium.*—*See* **39.043**(b). (Available from Difco Laboratories as Lactobacilli Agar Loy.)

(b) *Liquid culture medium.*—Dil. measured vol. of complete amino acid basal medium, **39.095,** with equal vol. H_2O. Add 10 ml portions to test tubes, plug with cotton (or screw cap tubes may be used), sterilize 15 min. in autoclave at 121–123°, and cool tubes as rapidly as practicable to keep color formation at min. Store in dark at ca 10°.

(c) *Liquid suspension medium.*—Dil. measured vol. appropriate basal medium (without amino acid being assayed), **39.095,** with equal vol. H_2O. Add 10 ml portions to test tubes, plug with cotton, sterilize 15 min. at 121–123°, and cool as

39.094 AMINO ACID STOCK SOLNS

Amino Acid	Weight	Initial Solvent	Final Volume	Final Concn
	g	ml	ml	mg/ml
(a) L-Alanine	5.000	900 H_2O	1000	5
(b) L-Arginine.HCl	12.095	400 H_2O	500	20
(c) L-Asparagine	5.000	400 H_2O	500	10
(d) L-Aspartic acid	10.000	100 1*N* NaOH	1000	10
(e) L-Cysteine.HCl	5.000	100 2*N* HCl	1000	5
(f) L-Cystine	5.000	100 2*N* HCl	1000	5
(g) L-Glutamic acid	10.000	100 1*N* NaOH	1000	10
(h) Glycine	10.000	400 H_2O	500	20
(i) L-Histidine.HCl	12.351	400 H_2O	500	20
(j) L-Hydroxyproline	5.000	150 H_2O	250	20
(k) L-Isoleucine	5.000	900 H_2O	1000	5
(l) L-Leucine	10.000	900 H_2O	1000	10
(m) L-Lysine.HCl	6.248	400 H_2O	500	10
(n) L-Methionine	5.000	900 H_2O	1000	5
(o) L-Norleucine	5.000	100 2*N* HCl	1000	5
(p) L-Phenylalanine	5.000	900 H_2O	1000	5
(q) L-Proline	5.000	150 H_2O	250	20
(r) L-Serine	5.000	900 H_2O	1000	5
(s) L-Threonine	5.000	900 H_2O	1000	5
(t) L-Tryptophan	5.000	50 1*N* NaOH	500	10
(u) L-Tyrosine	5.000	50 1*N* NaOH	500	10
(v) L-Valine	5.000	900 H_2O	1000	5

39.096 COMPOSITION OF BASAL MEDIUM

Ingredients (Stock solns, **39.093**)	250 ml	500 ml	750 ml	1 L
	ml	ml	ml	ml
L-Alanine	10	20	30	40
L-Arginine.HCl	5	10	15	20
L-Asparagine	5	10	15	20
L-Aspartic acid	5	10	15	20
L-Cysteine.HCl	5	10	15	20
L-Cystine	5	10	15	20
L-Glutamic acid*	25	50	75	100
Glycine	5	10	15	20
L-Histidine.HCl	5	10	15	20
L-Hydroxyproline	2.5	5	7.5	10
L-Isoleucine	10	20	30	40
L-Leucine	5	10	15	20
L-Lysine.HCl	5	10	15	20
L-Methionine	10	20	30	40
L-Norleucine	10	20	30	40
L-Phenylalanine	10	20	30	40
L-Proline	2.5	5	7.5	10
L-Serine	10	20	30	40
L-Threonine	10	20	30	40
L-Tryptophan	5	10	15	20
L-Tyrosine	10	20	30	40
L-Valine	10	20	30	40
(**b**) Adenine-guanine-uracil	5	10	15	20
(**e**) Vitamin Soln I	10	20	30	40
(**f**) Vitamin Soln II	10	20	30	40
(**c**) Salt Soln A	2.5	5.0	7.5	10
(**d**) Salt soln B	2.5	5.0	7.5	10
Dextrose, anhyd.				
with *S. faecalis*	5 g	10 g	15 g	20 g
with *L. plantarum* or *L. mesenteroides*	10 g	20 g	30 g	40 g
NaOAc, anhyd.				
with *S. faecalis*	3 g	6 g	9 g	12 g
with *L. plantarum* or *L. mesenteroides*	6 g	12 g	18 g	24 g

* For methionine assay use DL-glutamic acid since L-glutamic acid often contains methionine.

rapidly as practicable to keep color formation at min. Store in dark at ca 10°.

39.098 STOCK CULTURES OF TEST ORGANISMS

Prep. and test cultures as in **39.044**. Use following organisms:

(**a**) *Streptococcus faecalis*.—ATCC No. 9790. For use in assay of isoleucine, leucine, threonine, tryptophan, valine, arginine, and histidine.

(**b**) *Lactobacillus plantarum*.—ATCC No. 8014. For use in assay of isoleucine, leucine, methionine, phenylalanine, tryptophan, and valine.

(**c**) *Lactobacillus mesenteroides*.—ATCC No. 8042. For use in assay of lysine, methionine, phenylalanine, tyrosine, cystine, and histidine.

39.099 INOCULUM

Make transfer of cells from fresh stock culture of appropriate organism to sterile tube contg 10 ml liquid culture medium, **39.097**(**b**). Incubate 16–24 hr at 34 ±0.5°. Under aseptic conditions, centrifuge and decant supernatant. Suspend cells in 10 ml appropriate sterile suspension medium, **39.097**(**c**).

39.100 REFERENCE STANDARD SOLUTIONS OF AMINO ACIDS

Dry USP Reference Standard amino acid to constant wt over P_2O_5 and store in desiccator over P_2O_5. Prep. stock soln of each amino acid by weighing accurately amount designated in **39.101** in closed system and dil. to final vol. with H_2O. Prep. std working soln by dilg aliquot std stock soln to designated vol. with H_2O. Store std stock solns under toluene in dark at ca 10°. Prep. std working solns fresh for each assay.

39.101 PREPARATION OF REFERENCE STOCK AND STANDARD AMINO ACID SOLUTIONS

Amino Acid	Std Stock Soln mg	Std Stock Soln final vol. ml	Std Stock Soln concn mmg/ml	Std Working Soln aliquot ml	Std Working Soln final vol. ml	Std Working Soln concn mmg/ml
L-Arginine.HCl	121	500	200	5	200	5
L-Cystine (dissolve in 100 ml 1N HCl)	100	1000	100	15	500	3
L-Histidine.HCl.H_2O	135	1000	100	15	500	3
L-Isoleucine	100	1000	100	5	100	5
L-Leucine	100	1000	100			
(with *L. plantarum*)				5	100	5
(with *S. faecalis*)				10	100	10
L-Lysine.HCl	251	1000	200	15	200	15
L-Methionine	50	1000	50	10	250	2
L-Phenylalanine	100	1000	100	5	100	5
L-Threonine	100	1000	100	5	100	5
L-Tryptophan (dissolve in 100 ml 0.1*N* NaOH)	40	1000	40	5	250	0.8
L-Tyrosine (dissolve in 100 ml 0.1*N* NaOH)	100	1000	100			
(with *L. plantarum*)				5	250	2
(with *L. mesenteroides*)				10	250	4
L-Valine	100	1000	100			
(with *L. plantarum*)				10	200	5
(with *S. faecalis*)				10	100	10

39.102 CALIBRATION OF PHOTOMETER

Using inoculum, **39.099**, and std stock solns, **39.101**, proceed as in **39.047**, incubating 16–24 hr at 34 ±0.5°. In measurement, use basal medium, **39.095**, for dilg.

39.103 PREPARATION OF SAMPLE

(**a**) *Liquids containing free amino acids.*—Dil. with H_2O to concn required to obtain assay soln.

(**b**) *Dry or semi-dry materials.*—Add H_2O equal in ml to not <10 times dry wt in g of sample. Heat 10 min. in autoclave at 121–123°. Cool, dil. to vol., and filter if necessary to obtain assay soln.

39.104 ASSAY

Proceed as in **39.045,** using working std solns, **39.101,** assay soln, **39.103,** and basal medium **39.096** (omitting amino acid being assayed). Disregard references to titrimetric method and incubate 16–24 hr at 34 ±0.5°.

39.105 DETERMINATION

Proceed as in **39.048.**

39.106 CALCULATION

Proceed as in **39.049.**

BIOASSAY METHODS

Thiamine Hydrochloride (Vitamin B_1) (*17*)

Growth Method—Official

39.107 PRELIMINARY PERIOD

Thruout preliminary period each rat must be raised under immediate supervision of, or according to directions specified by, assayer, and kept on dietary regimen that provides for normal development in all respects, except that such dietary regimen must subsequently allow development of characteristic symptoms of vitamin B_1 deficiency (polyneuritis) in rats weighing 40–50 g, not >28 days of age, and subsisting on suitable vitamin B_1-deficient diet and H_2O for interval not >50 days.

39.108 DEPLETION PERIOD

Rat is suitable for depletion period when its age is not >28 days, if its body wt is >39 g and not >50 g, and if animal manifests no evidence of injury, or disease, or anatomical abnormality that might hinder growth or development. Thruout depletion period provide each rat with the vitamin B_1 test diet and H_2O *ad libitum,* and have no other dietary supplement available to animal. Thruout depletion period and until assay has been completed keep rats in cages provided with wire cloth bottoms, each mesh of which shall be not <8×8 mm.

39.109 ASSEMBLING RATS INTO GROUPS FOR ASSAY PERIOD

Assemble rats suitable for assay period into groups of at least 8 animals. For each assay material have one or more assay groups. In assay of each material, provide at least one control group and at least one reference group, but one control group and one reference group may be used for concurrent assay of more than one assay material.

For assembling rats into groups allow not >21

days. On any one day during interval of assembling rats into groups do not allow total number of rats that has been assigned to make up any one group to exceed by >2 the number of rats that has been assigned to make up any other group. When assembling of all groups has been completed, total number of rats in each group, and number of rats of one sex in each group, must be the same. Assign not >3 rats from one litter to one group. When assembling of all groups is complete, av. wt of rats in any one group on day beginning assay period must not exceed by >10 g the av. wt of rats in any other group.

39.110 ASSAY PERIOD

Rat is suitable for assay period provided depletion period has exceeded 10 days but not 30 days, and provided rat manifests evidence of vitamin B_1 deficiency characterized by stationary or declining wt. Thruout assay period keep each rat of control, reference, and assay groups in individual cage and provide with vitamin B_1 test diet and H_2O *ad libitum*. Thruout assay, administer reference material to each rat in reference group and administer assay material to each rat in assay group. In any one assay administer reference and assay material in same manner. During assay period maintain all conditions of environment as uniform as possible with respect to assay, reference, and control groups.

39.111 RECORDING OF DATA

On day beginning depletion period, record body wt of each rat. From seventh day of depletion period until beginning of assay period, record body wt of each rat at intervals not >3 days. During assay period, record body wt of each rat at intervals not >7 days.

39.112 VITAMIN B_1 POTENCY OF ASSAY MATERIAL

In detg vitamin B_1 potency of assay material calc. performance of rats of assay and reference groups on basis of difference between av. wt of each group at end of assay period and av. wt of same rats on day beginning assay period. Consider data from reference group valid for establishing vitamin B_1 potency of assay material only when $\frac{2}{3}$ or more of total number of animals comprising reference group have made individually, between beginning day of assay period and 28th day thereafter, increase in body wt equaling or >12 g and not >100 g. Data for assay group are not valid for establishing vitamin B_1 potency of assay material if av. wt of control group is greater at end of assay period than at beginning of assay period.

Total amount of assay material administered during assay period contains quantity of vitamin B_1 equal to or greater than total amount of vitamin B_1 administered to reference group during assay period if that quantity promotes in assay group av. gain in wt equal to or greater than av. gain in wt produced in reference group by administration of reference material.

If reference material and assay material are administered by inclusion in diet (option 4 below), make comparison of quantities of reference and assay material on basis of quantity of each contained in 100 g of supplement diets.

39.113 DEFINITIONS

As herein used, unless context otherwise indicates, *administer* means to supply reference material or assay material to each rat in any one of following manners:

(1) By injecting parenterally; (2) by placing in mouth of animal daily; (3) by placing before animal daily in dish separate from vitamin B_1 test diet; and (4) by replacing equal wt of sucrose in vitamin B_1 test diet and intimately mixing material with diet.

Assayer means individual immediately responsible for interpretation of assay; *assay group* means group of rats to which assay material is administered during assay period; *assay material* means material under examination for vitamin B_1 content; *assay period* means interval in life of rat between last day of depletion period and 29th day thereafter; *assemble* means procedure by which rats are selected and assigned to groups for purposes of feeding, care, and observation; *control group* means group of rats receiving no assay or reference material during assay period; *daily* means 6 days of each week of assay period; *depletion period* means interval in life of rat between last day of preliminary period and first day of assay period; *reference group* means group of rats receiving reference material during assay period; *reference material* means USP Vitamin B_1 Reference Standard; *stationary or declining wt* means condition of rat when body wt on any given day is equal to or less than body wt on fifth day prior to given day; *vitamin B_1 test diet* means uniform mixt. that has not been compounded for >14 days, of following food materials in proportions designated:

39.114 VITAMIN B_1 TEST DIET

	per cent
Sucrose	60
Casein (1)	18
Salt Mixture (2)	4
Autoclaved Yeast (3)	5
Autoclaved Peanuts (4)	10
Treated Liver Extract (5)	1
Cod Liver Oil (USP)	2
Pyridoxine	0.0002

(1) Free from demonstrable traces of vitamin B_1.

(2) Either USP salt mixt. or salt mixt. having essentially same proportions of the elements. Prep. USP XVI salt mixt. as follows: Grind in mortar portion of 139.3 g NaCl with 0.79 g KI. Similarly grind together remainder of the NaCl with 389.0 g KH_2PO_4, 57.3 g $MgSO_4.7H_2O$, 381.4 g $CaCO_3$, 27.0 g $FeSO_4.7H_2O$, 4.01 g $MnSO_4.H_2O$, 0.548 g $ZnSO_4.7H_2O$, 0.477 g $CuSO_4.5H_2O$, and 0.023 g $CoCl_2.6H_2O$, adding finally the NaCl-KI mixt. Reduce entire mixt. to fine powder.

(3) Autoclave 5 hr in steam at 15 lbs pressure, layers of dried yeast not >6 mm deep; then dry at temp. not >65°.

(4) Crush unroasted, shelled, No. 1 Virginia peanuts in food chopper; autoclave 5 hr in steam at 15 lb pressure in layers not >12 mm deep; then dry at temp. not >65°. Incorporate in basal diet by grinding with requisite quantity of sucrose.

(5) Dissolve 100 g liver ext. in 1 L 0.6% $NaHSO_3$ soln. (Salt used should be tested to insure bisulfite content.) Let soln stand 24 hr in well-stoppered bottle; then acidify with HCl to pH 1.5, distill under reduced pressure at temp. not >50° until vol. is reduced one-half, and finally dry on 1500 g vitamin B_1-free casein at temp. not >65°. For each 100 g test diet, use quantity of this mixt. equiv. to 1 g actual dried liver ext., and consider quantity of casein added with this ext. as part of total 18 g casein in 100 g diet.

Vitamin D (*18*)—Official

(Not applicable to products offered for poultry feeding.)

39.115 DEFINITIONS

Assay group means group of rats to which assay sample (vitamin D sample) is administered during assay period. *Assay sample* means sample under examination for vitamin D potency. *Assay soln* means soln of sample in oil prepd for feeding after saponification procedure. *Assay period* means interval in life of rat between last day of depletion period and eighth or eleventh day thereafter. *Assemble* means procedure by which rats are selected and assigned to groups for purposes of feeding, care, and observation. *Daily* means each of first 6 or 8 days of assay period. *Depletion period* means interval in life of rat between last day of preliminary period and first day of assay period. *Dose* means quantity of reference oil or of assay milk or other supplement to be fed daily to rat during assay period. *Feed* means make readily available to rat or administer to rat by mouth. *Group* means 7 or more rats maintained on same required dietary regimen during assay period. *Preliminary period* means interval in life of rat between seventh day after birth and first day of depletion period. *Reference oil* means USP Vitamin D Reference Standard.

39.116 REAGENTS

(a) *Ground gluten.*—Clean, sound product made from wheat flour by almost complete removal of starch, contg not >10% H_2O and, calcd on H_2O-free basis, not <14.2% N, not >15% N-free ext. (using protein factor 5.7), and not >5.5% starch (detd by diastase method, **22.045**).

(b) *Reference oil.*—USP Vitamin D Reference Standard.

(c) *Cottonseed oil.*—USP grade meeting following addnl requirements: Saponify 10 g oil as in **39.120**, and dissolve unsaponifiable residue in 10 ml petr. ether. In sep. container place 0.4 ml $FeCl_3$ soln (1+1000) and 12 ml of soln of α,α-dipyridyl in absolute alcohol (1+6000), mix, and 5 min. later read absorbance in 1.0 cm cell at 520 mμ, using suitable spectrophotometer, against absolute alcohol. Then add 0.2 ml of soln of unsaponifiable residue in petr. ether to entire colored soln, and after 5 min. read absorbance. Difference between first and second absorbance values is not <0.125.

(d) *Rachitogenic diet.*—Mix 76% whole yellow corn, ground to pass No. 30 sieve; 20% gluten, ground to pass No. 30 sieve; 3% $CaCO_3$; and 1% NaCl.

39.117 PRESERVATION OF SAMPLE

Store samples so as to minimize exposure to heat, light, and air. Milk samples must be delivered in original container immediately after collection or be stored under refrigeration in iced container until delivered. After delivery to assayer, milk must be preserved in its homogeneous state by refrigeration at temp. of not >10° for not >10 days, or be preserved for not >30 days by addn of 2 drops 10% HCHO soln to 1 quart of milk in addn to refrigeration at temp. of not >10°. Evapd and reconstituted milk must be preserved in same manner as fluid milk. Soured or curdled sample is unsuitable for assay purposes. Sample of dried milk, after being opened by assayer, must be preserved by refrigeration at temp. of not >10°.

39.118 SAMPLE

Sample shall consist of not <10 g food, 10 capsules or tablets, or sufficient vol. of liquids to satisfy needs of entire assay.

If amount of vitamin D in sample is such that aliquot to be fed contains <5 mg P, sample may be fed directly. If aliquot contains >5 mg P, sample must be saponified.

All manipulations and dilns of vitamin sample must be made with materials known to be free of vitamin D.

39.119 PREPARATION OF SAMPLE FOR DIRECT FEEDING

(**a**) *Feeds and tablets.*—Grind weighed sample thoroly. Promptly weigh aliquot of ground powder and grind it again with equal wt of edible vegetable oil. To this add such quantity of powd. sucrose that assay dose will be contained in 1–2 g. Mix thoroly by grinding again and proceed as in assay period, **39.125**.

(**b**) *Capsules.*—Open weighed capsules and transfer contents as completely as possible into container. Thoroly mix combined contents and promptly weigh aliquot. Proceed as in (**a**). Obtain sample wt by subtracting wt empty ether-washed capsules from total wt of capsules.

(**c**) *Oils.*—Add amount of edible vegetable oil that will produce diln contg assay dose in vol. equal to vol. of reference diln.

(**d**) *Water-miscible liquids.*—Dil. as for oils, using H_2O, glycerol, or propylene glycol to facilitate feeding.

39.120 PREPARATION OF SAMPLE BY SAPONIFICATION

Weigh sample and transfer to saponification flask. (For milk, see **39.121**.)

In case of capsules or tablets, place not <10 in small reflux flask, add 10 ml H_2O, and heat on steam bath ca 10 min. Crush each capsule or tablet with blunt glass rod and warm 5 min. more. Add 2 ml cottonseed oil and vol. of KOH (50% w/w) soln representing 2.5 ml for each g total wt of sample plus cottonseed oil, but not <15 ml. Add 50 ml alcohol and reflux vigorously 30 min. in 100° bath. Cool soln and transfer to Squibb-type separator, using 50 ml H_2O. Ext. with four 30 ml portions of peroxide-free ether (USP anesthesia ether is suitable), using more H_2O or small portions of alcohol to break any emulsions that may form. Wash combined ether exts 4 times with H_2O as follows: (1) 100 ml with very gentle swirling; (2) 100 ml with gentle swirling; (3) 50 ml with gentle shaking; (4) 50 ml with vigorous shaking. Dry ether ext. with two 75 ml portions satd NaCl soln, shaking vigorously both times. Transfer ether ext. to beaker and evap. on steam bath to convenient vol. If H_2O is present, dry with 3–5 g anhyd. Na_2SO_4. Decant into weighed container, rinse beaker and Na_2SO_4 with 3–5 addnl portions ether, and combine all washings in weighed container. Evap. ether on steam bath until no ether odor is detectable, and weigh fat. Multiply by 1.10 to det. vol., and add amount of edible vegetable oil that will produce convenient final diln for feeding. Mix thoroly (magnetic stirrer is desirable).

39.121 PREPARATION OF MILK SAMPLES

Proceed as in **39.120** with the following special modifications: Use 50–100 ml alcohol and 10 g KOH pellets per 100 ml sample. Swirl until all KOH dissolves. Reflux 40–60 min. (Placing several short pieces of glass stirring rod in the saponification flask and use of oil or H_2O bath at 100° is recommended to minimize bumping and scorching of sample.) Use 50–100 ml ether for first extn. Only small part of butterfat is saponified, but fat may be fed without affecting results. Where unusually large amount (>0.5 g) of fat would have to be fed in every dose, ext. from which ether has been evapd may be resaponified as in **39.120**.

39.122 PRELIMINARY PERIOD

Thruout preliminary period each rat must be raised under immediate supervision of, or according to directions specified by, assayer. Thruout preliminary period, keep rats on dietary regimen that provides for normal development in all respects, except to limit supply of vitamin D to such degree that rats, weighing 40–60 g at age of 21–30 days, and subsisting 18–25 days on suitable rachitogenic diet, show evidence of severe rickets.

39.123 DEPLETION PERIOD

Rat is suitable for depletion period when its age is not >30 days, and its body wt is >44 g but not >60 g, provided it shows no evidence of injury, disease, or anatomical abnormality that might hinder growth and development. Thruout depletion period provide each rat with rachitogenic diet, and H_2O or USP H_2O *ad libitum*, and permit no other dietary supplement to be available.

39.124 ASSEMBLING RATS INTO GROUPS FOR ASSAY PERIOD

Assemble rats that are suitable for assay period into groups. For each sample provide one or more assay groups. In assay of one sample at least one reference group must be provided, but this reference group may be used for concurrent assay of more than one assay sample. (Where 2 reference groups are desired, dose levels must be selected so that ratio of higher to lower dose is not <1.5 or >2.5. Dosage levels for samples based upon single assumed potency for each sample may be equiv. to reference levels or at mid-level equal to square root of product of the 2 dosage levels of the reference.) On any one day during interval of assembling rats into groups, total number of rats assigned to make up any one group must not exceed by >2 the number of rats that have been assigned to make up any other group. When assembling of all groups is completed, total number of rats in each group must be same. Assign not >3 rats from 1 litter to assay group unless equal number of rats from same litter is assigned to

reference group. There must be enough animals in each group to meet requirements specified in **39.128.**

39.125 ASSAY PERIOD

Rat is suitable for assay period provided depletion period is >18 days but not >25 days, and provided rat shows evidence of rickets characterized by distinctive wobbly rachitic gait and enlarged joints. Presence of rickets may also be established by examination of leg bone of one member of litter by "line test," **39.126,** or by X-ray examination of animals selected for assay. Keep each rat in individual cage, provided with the rachitogenic diet and H_2O *ad libitum.* On any calendar day of assay period, assay and reference groups must receive rachitogenic diet compounded from same lots of ingredients.

Following optional methods of feeding reference oil soln and sample soln are permissible, but both reference oil soln and sample soln must be fed according to same method in any 1 assay. Supplements may be fed on first day of assay period, or in equal portions on first, third, and fifth days, or on first and third day, or on first and fourth days of 7 day or 10 day assay period, or on first 6 days of 7 day assay period, or on first 8 days of 10 day assay period; supplements may be fed admixed with quantity of basal ration that will be consumed within first 5 days of 7 day assay period or within first 8 days of 10 day assay period. In each case make unsupplemented ration available during remainder of assay period.

Feed quantity of reference oil found by experience to cause extent and degree of calcification of rachitic metaphysis equiv. to 4 on line test chart. Feed that quantity of sample soln which is calcd to contain, on basis of claimed or assumed potency, same number of units of vitamin D as contained in quantity of reference oil fed.

After assay period kill each rat and examine one or more leg bones for healing of rachitic metaphysis according to "line test," **39.126.**

Reference oil may be dild with edible vegetable oil free from vitamins A and D before being fed. Dild oil must be stored in dark at temp not $>10°$ for not >30 days. Do not feed >0.2 ml of dild oil as daily dose. During assay period keep all conditions of environment (particularly with reference to physiologically active radiations) as uniform as possible with respect to assay and reference groups.

39.126 LINE TEST

Make line test on proximal end of tibia or distal end of radius or ulna. Remove end of desired bone from animal and clean of adhering tissue. Make longitudinal median section thru end of bone with clean, sharp blade to expose plane surface thru junction of epiphysis and diaphysis. In any one assay use same bone of all animals and section thru same plane. Rinse both sections of bone in H_2O and immerse in 2% $AgNO_3$ soln 1 min. Then rinse sections in H_2O and expose sectioned surfaces in H_2O to daylight or other source of actinic light until calcified areas have developed clearly defined stains without marked discoloration of uncalcified areas. Record immediately extent and degree of calcification of rachitic metaphysis of every section.

Staining procedure may be modified to differentiate more clearly between calcified and uncalcified areas. Suitable alternative procedure is to take freshly sectioned bone and proceed as follows: (1) Soak in ether-acetone mixt. $(3+1)$ at least 5 min. (at this stage, after bone sections are dry, they may be mounted for convenience and ease of handling on std microscope slides with aid of rubber cement and remainder of procedure performed in Coplin staining jars); (2) soak in alcohol 10 min.; (3) soak in acetone 10 min.; (4) soak 40 min. in H_2O which is completely changed after 1, 10, 20, and 30 min.; (5) stain with 2% $AgNO_3$ soln 60 sec.; and (6) wash 40 min. with H_2O in dark with complete changes after 1, 10, 20, and 30 min. Expose stained sections in H_2O to daylight or other source of actinic light until stains have developed.

Score degree of calcification of rachitic metaphysis in each rat according to scale shown in Fig. 87. Because lines pictured in chart differ somewhat from line of healing being scored, it is necessary to visualize calcification as if it were compact and continuous in comparing it with appropriate figure in chart. Use of chart is illustrated by accompanying photographs of actual sections of radii, Fig. 88.

39.127 RECORDING OF DATA

On day beginning assay period and on seventh or tenth day thereafter, depending on duration of assay period, record body wt of each rat. Keep record of quantity of rachitogenic diet consumed /rat during assay period. Assign numerical values to extent and degree of calcification of rachitic metaphyses of bones examined by line test by comparison with line test chart so that it is possible to average performance of each group.

39.128 POTENCY OF ASSAY SAMPLE

Consider data from reference or sample group valid for establishing vitamin potency of assay sample only when 2/3 or more, but not <7 rats individually show extent and degree of calcification of rachitic metaphysis equal to or >0.5 and equal to or <8.0 on line test chart.

When av. response of assay group is less than, equal to, or more than that of reference group,

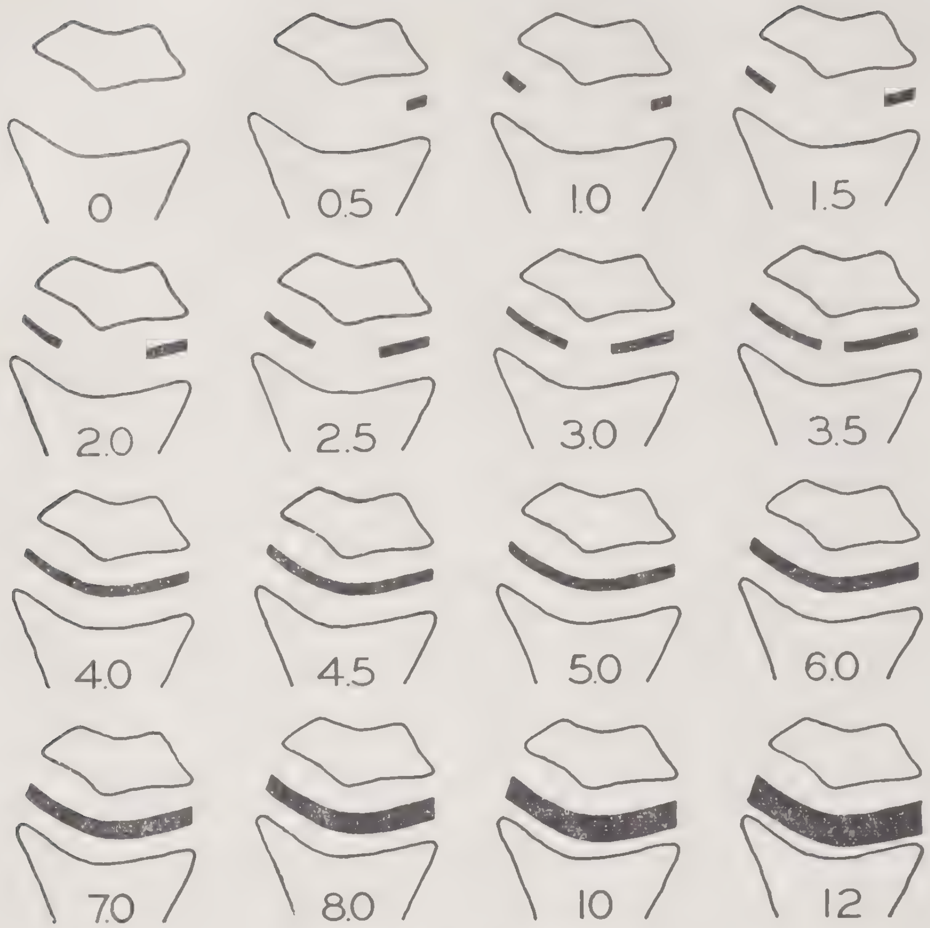

FIG. 87.—LINE TEST CHART

vitamin D content of sample aliquot is respectively less than, equal to, or more than the vitamin D content of the reference aliquot fed during the assay period.

Consider data from rat valid for establishing av. performance of group only when wt of rat at termination of assay period equals or exceeds wt on beginning day of assay period, and only when rat has consumed av. of not <4 g of rachitogenic diet daily during assay period and has consumed each prescribed dose of assay soln within 24 hr from time it was fed.

39.129 CALCULATION

When above conditions have been met and response of assay group is equal to that of reference group, calc. vitamin D potency of assay sample as follows:

Let R = total number USP units of vitamin D fed each rat in reference group during assay period; and M = fraction of assay sample unit fed each rat in assay group during assay period. Then: R/M = USP units vitamin D/unit of sample.

Vitamin D in Poultry Feed Supplements (*19*)—Official

(Applicable to fish and fish liver oils and their exts, and to materials used for supplementing vitamin D content of feeds. Not applicable to irradiated ergosterol products or to irradiated yeast unless recommended for poultry. This assay is comparison, under conditions specified, of efficacy of product under assay with that of USP Vitamin D Reference Standard in controlling ash content of bones of growing chicks.)

39.130 BASAL RACHITIC RATION

The basal ration is uniform mixt. in proportions designated of following ingredients, which have been finely ground:

	per cent
Yellow corn, ground	58
Wheat flour middlings or wheat gray shorts	25
Casein, crude, domestic, acid pptd	12
Calcium phosphate, pptd	2
Salt, iodized (0.02% KI)	1
Yeast, non-irradiated (7% min. N)	2

To each kg of above mixt. add 0.2 g $MnSO_4.4H_2O$.

FIG. 88.—PHOTOGRAPHS OF RADII SECTIONS SCORED ACCORDING TO LINE TEST CHART. FOR ILLUSTRATIVE PURPOSES ONLY; SHOULD *NOT* BE USED AS SCORING SCALE

39.131 DETERMINATION

Provide cages with screen bottoms and keep chicks away from sunshine or other source of actinic light that may influence calcification. Keep cages in rooms in which wide variations in temp. are prevented (constant temp. preferred). Unless temp. of room is adequately controlled, provide each cage with suitable elec. heating device. Start all birds to be used in one assay on same day and keep all conditions of environment uniform for all groups in assay.

Make assay on groups of 1- or 2-day-old white Leghorn chicks as specified below. Provide for one or more negative control groups that receive no vitamin D, three or more positive control groups that receive graduated levels of vitamin D from USP Vitamin D Reference Standard, and one or more assay groups for each product to be assayed. Have positive control and assay groups consist of not <20 birds each, and negative control group consist of not <10 birds.

Prepare rations for all groups in assay from one batch of basal ration. Add Reference Standard to basal ration in such quantities as to produce measurable increase in % bone ash above that obtained in negative control group (it is not possible to make comparisons if max. bone ash is obtained). Add assay product to basal ration in such quantities as to permit direct comparison in response of assay and positive control groups.

To basal ration of negative control group add corn oil equal in quantity to max. quantity of oil

fed to any group in assay, and add corn oil to rations of other groups until total quantity of corn oil and oil contg vitamin D is equal to quantity of corn oil added to ration of negative control group.

Feed chicks in respective groups prescribed ration and H_2O (natural or distd) *ad libitum* 21 days. Discard all chicks that show abnormality or disease not related to vitamin D deficiency. At least 15 chicks must remain in each reference or assay group used in calcg vitamin D potency of assay product.

Kill chicks; remove left tibia of each bird and clean of adhering tissue. (To facilitate removal of adhering tissue, bones may be placed in boiling H_2O not >2 min. Bones may be preserved in alcohol for extn.) Completely ext. bones with suitable fat solvent or solvents (20 hr with hot alcohol followed by 20 hr with ether is suitable, and bones should be crushed to facilitate extn). Dry extd bones to constant wt at 95–100° under pressure <100 mm Hg (ca 5 hr), cool in desiccator, and weigh. Ash H_2O- and fat-free bones from each group of birds in muffle furnace to constant wt at any given temp. between 450 and 550°, or if preferred, 1 hr at ca 850°. (Ash detn may be made on individual bones if desired.) Use consistently thruout any one assay the specific procedure adopted for extg, drying, and ashing of the bones.

39.132 INTERPRETATION OF RESULTS

One international chick unit of vitamin D is equal in biological activity to one unit vitamin D in USP Vitamin D Reference Standard in this method of assay. Product under assay meets its declared vitamin potency in international chick units of vitamin D if % ash in H_2O- and fat-free bone produced in assay groups by given number of units of vitamin D is equal to or is greater than % ash produced by same number of units of vitamin D from USP Reference Standard.

Biological Evaluation of Protein Quality (*20*)—First Action

(Applicable to materials contg >9% protein (N×6.25))

39.133 REAGENTS

(a) *ANRC reference casein.*—Available from Sheffield Chemical Co., Norwich, N. Y.

(b) *Salt mixture USP XVI.*—Or salt mixt. having essentially the same proportions of the elements. *See* **39.114**(2).

(c) *Vitamin mixture.*—

	Mg/100 g ration
Vitamin A (dry, stabilized)	2000 (IU)
Vitamin D (dry, stabilized)	200 (IU)
Vitamin E (dry, stabilized)	10 (IU)
Menadione	0.5
Choline	200
p-Aminobenzoic acid	10
Inositol	10
Niacin	4
Ca D-pantothenate	4
Riboflavin	0.8
Thiamine.HCl	0.5
Pyridoxine.HCl	0.5
Folic acid	0.2
Biotin	0.04
Vitamin B_{12}	0.003
Dextrose, to make	1000

(d) *Cottonseed oil.*

(e) *Cellulose.*—Cellu Flour, Solka Floc, or equiv.

(f) *Protein evaluation basal diet.*—

Sample	A^*
Cottonseed oil	$8 - \frac{A \times \%\text{ ether extract}}{100}$
Salt mixture USP	$5 - \frac{A \times \%\text{ ash}}{100}$
Vitamin mixture	1
Cellulose	$1 - \frac{A \times \%\text{ crude fiber}}{100}$
Water	$5 - \frac{A \times \%\text{ moisture}}{100}$
Sucrose, to make	100

$$*A = \frac{9.09 \times 100}{\%\text{ Protein of Sample } (N \times 6.25)}$$

All percentage figures refer to sample. Proximate analysis is needed to adjust diet so that all comparisons between samples and reference material shall be made with diets having same content of N, fat, ash, moisture, and crude fiber. These suggested levels of fat, ash, moisture, and crude fiber are desirable whenever proximate analysis of sample permits.

39.134 EXPERIMENTAL ANIMALS

Laboratory rats, males, shall be from same colony, and maintained during period before weaning upon diet and under environmental conditions that will provide for normal development in all respects; weaned not <21 days of age and not >28 days of age; range of individual rat wts among animals used shall be not >10 g.

39.135 ASSAY GROUPS

Assemble rats into groups of at least 10 rats. In assay of each material provide 1 group that will receive ANRC reference casein. One reference

casein group may be used for concurrent assay of >1 assay material. When assembling of all groups is complete, total number of rats in each group must be the same, and av. wt of rats in any 1 group on day beginning assay period must not exceed by >5 g av. wt of rats in any other group.

39.136 ASSAY PERIOD

Thruout assay period keep each rat in individual cage and provide with appropriate assay diet and H_2O *ad libitum*. During assay period maintain all conditions of environment as uniform as possible with respect to each of groups being compared to ANRC reference casein. Record body wt of each rat on beginning day of assay period and body wt and food intake of each rat at regular intervals, not >7 days, and on 28th day after beginning of assay period.

39.137 CALCULATION AND TABULATION OF RESULTS

Calc. av. 28 day wt gain and protein intake per rat for each group. Calc. Protein Efficiency Ratio (PER) (wt gain/protein intake) for each group. Det. ratio×100 of PER for each assay group to PER for ANRC casein reference group. Tabulate 28 day weight gains, protein intake, PER, and ratio×100 of sample PER to ANRC Reference Casein PER for each assay group. Report protein quality of sample as ratio×100 of sample PER to ANRC reference casein PER.

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(*4*) Ind. Eng. Chem., Anal. Ed. **13,** 600(1941); **15,** 18(1943); **16,** 513(1944); **19,** 170(1947); J. Biol. Chem. **164,** 2(1946); J. Assoc. Offic. Agr. Chemists **25,** 573, 886(1942); **26,** 77(1943); **27,** 542(1944); **28,** 563(1945); **29,** 18(1946); **30,** 412(1947); **31,** 459, 621, 623, 776(1948); **32,** 480, 766, 775, 804(1949); **33,** 647(1950); **34,** 387, 460(1951); **35,** 736, 826(1952); **36,** 857(1953); **37,** 753, 756, 880, 887, 894(1954); **38,** 694(1955); **39,** 139(1956); **40,** 865(1957); **41,** 600(1958); **42,** 528(1959).

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(*7*) Ibid. **40,** 843(1957); **41,** 603(1958).

(*8*) Ibid. **23,** 346(1940); **24,** 413(1941); **25,** 459(1942); **26,** 81(1943); **27,** 540(1944); **30,** 392(1947); **31,** 701(1948); **32,** 108, 461(1949); **33,** 88, 632(1950); **37,** 770(1954); **43,** 42(1960).

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(*12*) Science **100,** 295(1944); J. Biol. Chem. **157,** 303(1945); **163,** 447, 449(1946); Ann. N. Y. Acad. Sci. **48,** 261(1946); Analyst **72,** 84(1947); J. Assoc. Offic. Agr. Chemists **31,** 466(1948); **32,** 464(1949); **33,** 633(1950); **39,** 172(1956); **40,** 855, 856(1957); **41,** 61, 587, 591(1958); **42,** 529(1959).

(*13*) J. Assoc. Offic. Agr. Chemists **27,** 105(1944); **30,** 82(1947); **32,** 110, 479(1949); **39,** 172(1956); **40,** 856(1957); **41,** 61, 587(1958); **42,** 529(1959).

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(*20*) Ibid. **43,** 38(1960).

40. Radioactivity

40.001 Qualitative Test—Official
(Applicable to solids)

Charge alpha ray electroscope (preferably of Lind type) to bring leaf to suitable position on scale in microscope. Close door and record position of leaf on scale at frequent intervals until rate of fall of leaf is constant. Calc. rate of fall of leaf in divisions/min., designating figure obtained as natural leak of instrument for that particular detn.

Place convenient portion of sample on pan of electroscope, close door, recharge leaf system, and record rate of fall of leaf in divisions/min. over same range of scale as before, until rate becomes constant, recharging if necessary. Rate of fall in excess of natural leak of instrument shows that sample is radioactive.

QUANTITATIVE METHODS

Emanation or Radon Method (1)—Official

(Applicable only to Ra in quantities $<10^{-9}$ g. This limit is arbitrary and depends on particular equipment used and accuracy required.)

40.002 REAGENTS

(All reagents should be free from Ra and Rn)

(**a**) *Nitrogen.*—Use tank N that has stood at least 30 days.

(**b**) *Radium std soln.*—Use 100 ml std soln contg 10^{-9} g Ra and 100 ml blank rinsing soln issued by NBS.

(**c**) *Phosphorus pentoxide.*—Resublimed.

(**d**) *Calcium chloride.*—Fused.

(**e**) *Mercury.*—Redistd.

40.003 APPARATUS

Use all-glass app., Fig. 89. Provide several units for storing sample (unit includes Nos. *2*, *4*, *5*, *7*, *8*, *10*, and *11*).

Nos. *1* and *2* are 300 ml Pyrex flasks. Flask No. *1* has tube sealed on to insert std soln. Nos. *3* and *4* are 8″ condensers with ca $\frac{3}{4}$″ inner tubes; 6 mm Pyrex tubing is used for all connections. No. *5* is ₸ 24/40 joint; *7* and *10* are ₸ 7/25 joints; *8*, *11*, *17*, *18*, and *23* are 2 mm bore, well-ground, 2-way Pyrex stopcocks. Nos. *6*, *9*, *14*, *16*, and *21* are 2 mm bore, 3-way Pyrex stopcocks carefully lubricated with min. of grease. Use care to keep bores free of grease.

No. *12* is $CaCl_2$ drying tube of $\frac{3}{4}$″ Pyrex tubing 10″ long with glass wool plug at each end. (P_2O_5 may be substituted for the $CaCl_2$.) No. *13*, P_2O_5 tube to remove last traces of H_2O, has same dimensions and construction as $CaCl_2$ tube, but is filled with mixt. of glass beads and the P_2O_5. No.

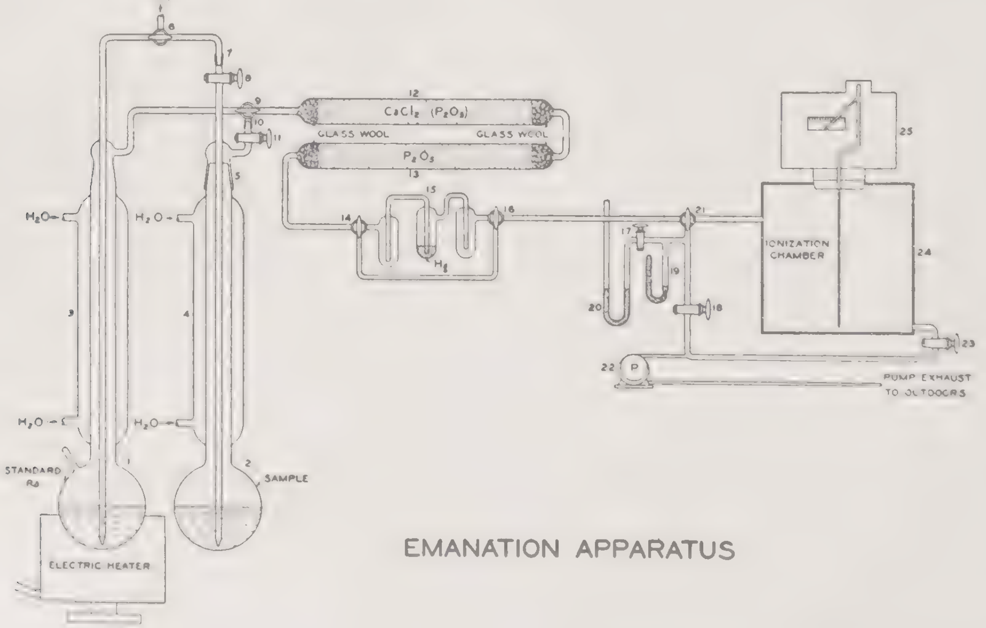

FIG. 89.—APPARATUS FOR DETERMINING RADIOACTIVITY BY EMANATION METHOD

15 is Hg trap and bubble counter; have center tube dip just below surface of the Hg. Use $\frac{3}{4}''$ tubing with vol. of ca 20 ml for each tube. No. *19* is closed-end manometer to measure vac. of system. No. *20* is open-end manometer with ca 10 cm Hg in each arm; it is used only near end of reflux to bring system to atmospheric pressure. No. *22* is oil pump capable of producing vac. of at least 0.5 mm.

No. *24* is ionization chamber, ca 2.8 L. It consists of brass cylinder, 6″ high×6″ diam., provided with vac.-tight brass inlet tube near top and brass outlet tube near bottom. Use care that no solder gets into chamber. Wall of chamber should be grounded. Electrode is $\frac{1}{16}''$ brass wire, insulated from chamber by being threaded thru tapered amber plug and connected with short lead to both charging device and leaf or fibers of electroscope or electrometer.

No. *25* may be either sensitive electroscope or electrometer. Either leaf- or filament-type electroscope may be used, and electrometer may be single- or double-filament type. To make electrical system as sensitive as possible, have electroscope or electrometer mounted as close to electrode as possible, preferably on top of ionization chamber. There should also be provided electronic rectifying device capable of maintaining potential of 200–500 volts. Negative terminal is connected by suitable switch to electrode of chamber and support of leaf or fibers of electroscope. Positive terminal is grounded.

40.004 PREPARATION OF SAMPLE

Conduct all detns in acid soln (either HCl or HNO_3) and free from sulfate and SiO_2. Soln should be clear and contain no ppt or suspended matter.

A. *Samples completely soluble in acids:*

(1) *Solid or semi-solid form.*—Add 50 ml HNO_3 (1+9) and boil several min. If residue remains, add 50 ml HCl (1+9) and again boil. (Do not apply this treatment to samples contg grease, such as face creams, physical appearance of which indicates that they are insol. in aq. solns.)

(2) *Liquid form (clear liquid, turbid liquid, or liquid containing suspended matter).*—Add 50 ml HNO_3 (1+9) to 1–10 ml sample, boil several min., and examine carefully for opalescence. If portion of sample remains undissolved, add 50 ml HCl (1+9) and again boil. If clear solns are obtained, proceed as in **B** (**c**).

B. *Samples wholly or partly insoluble in acids:*

(**a**) *Preliminary treatment:*

(1) *Solids.*—If sample is not in powder form, grind to fine powder; ignite weighed portion in porcelain dish in muffle at 500–600°, avoiding fusion; and proceed as in (**b**).

(2) *Semi-solids.*—Ignite quite rapidly in muffle weighed portion of sample in porcelain dish avoiding fusion. (Heating too slowly or heating in open may cause sample to creep over edge of dish.) Proceed as in (**b**).

(3) *Liquids immiscible with H_2O.*—Evap. weighed or measured portion of sample to dryness, or as nearly so as possible on steam bath, and dry carefully on hot plate. Ignite residue in muffle, avoiding fusion. Proceed as in (**b**).

(4) *Liquids containing material insoluble in HNO_3 (1+9).*—Digest sample or suitable portion of it with HNO_3 (1+9). Filter into 300 ml Florence flask and wash residue thoroly with hot H_2O. Proceed as in (**b**)(1), beginning "Ignite washed residue in Pt dish . . . "

(**b**) *Treatment of ash:*

(1) Digest ash obtained in (**a**) with HNO_3 (1+9) on steam bath. Filter into Florence flask and wash thoroly with hot H_2O. (300 ml flask is usually most suitable, even if it is necessary to conc. filtrates by boiling.) Ignite washed residue in Pt dish and cover residue with few ml H_2O and 5–10 ml HF. Evap. to dryness on steam bath. Add H_2O and few ml of the HNO_3, digest on steam bath, filter into Florence flask, and wash with H_2O. Ash filter paper in Pt dish, and add 5–10 ml H_2O and 1 ml of the HNO_3. Examine carefully for any insol. material; if none is found, add soln directly to Florence flask, rinsing dish several times with H_2O and adding washings to flask. Proceed as in (**c**).

(2) If insol. residue that does not contain $BaSO_4$ remains, proceed as follows: Ignite insol. residue in Pt dish and fuse with 5–10 times its wt of fusion mixt. consisting of equal wts of K_2CO_3 and anhyd. Na_2CO_3. Cool, and cover with watch glass. Neutralize fused mass with HNO_3 (1+9), using drop of phthln to indicate when soln is acid. Heat on steam bath, add few ml excess of the HNO_3, and boil carefully. Filter soln into the Florence flask and wash thoroly. Ignite insol. residue in Pt dish and proceed as in (**b**)(1), beginning "cover residue with few ml H_2O and 5–10 ml HF."

(3) If insol. residue contains appreciable $BaSO_4$, proceed as follows: Ignite insol. residue in Pt crucible, mix, and fuse with 5–10 times its wt of fusion mixt. consisting of equal wts K_2CO_3 and anhyd. Na_2CO_3. Cool, boil residue with little H_2O until thoroly disintegrated, and filter. Since this soln contains SO_4, do not mix with acid filtrate obtained in (**b**)(1). Wash residue with hot 1*N* Na_2CO_3 until filtrate gives no test for SO_4, and then with little H_2O. Dissolve washed residue ($Ba\text{-}RaCO_3$) carefully with HNO_3 (1+1). If

clear soln results, combine with original acid filtrate. If insol. residue remains, proceed as in (**b**)(1), beginning "Ignite washed residue . . ." Combine with original acid filtrates.

(**c**) *Final preparation of clear solns:*

Evap. clear acid solns obtained in (**a**) or (**b**) to dryness in Pt dish. Add 10 ml HCl (1+4) and again evap. to dryness. Repeat HCl evapn.

Take up residue in 25 ml of the HCl, warm, and filter into 200 ml vol. flask, washing dish and paper well with hot H_2O until vol. in flask is 100–125 ml. Add 40 ml 10% $BaCl_2.2H_2O$ soln and dil. to vol. Clear soln should result.

40.005 DETERMINATION

Keep stopcock *17* closed at all times except when system is at or near atmospheric pressure. With stopcocks *9* and *17* closed and *14*, *16*, and *21* open, evacuate system to pressure of ca 1 mm as shown on manometer *19*. Close stopcock *18*, shut off pump, and let system stand 1 hr. Pressure should remain <5 mm.

(1) *Inserting std.*—Place into flask *1*, thru attached tube, the 10^{-9} g Ra std soln, **40.002**(**b**). Rinse into flask with the blank soln.

(2) *Inserting sample.*—Insert in flask *2* subdivision of clear soln of sample obtained, **40.004**(**c**), that will produce accurately measurable increase in rate of discharge of the electroscope, by disconnecting flask and condenser at joint *5*, and adjust to ca 200 ml with boiled HCl (1+19) contg 2% $BaCl_2$. Then lubricate joint with P_2O_5 at its outer periphery, leaving joints *7* and *10* dry and assemble app., taking care to have joints tight.

(3) *De-emanation of std.*—Manipulate stopcocks *6*, *9*, *14*, *16*, *18*, and *21* so that gentle stream of N (ca 3 bubbles/sec. as shown in Hg trap) passing thru soln in flask *1* will carry the Rn thru pump exhaust to outdoors without entering ionization chamber. Heat soln to boiling with elec. heater and reflux 20–25 min. in gentle stream of N. Remove elec. heater, close cock *6* first and then *9*, noting exact time that stopcock *9* is closed. This seals off std in flask *1*, and as soln cools slight vac. set up in this portion of system minimizes loss of emanation.

(4) *De-emanation of sample.*—Treat sample in flask *2* exactly as for std, taking care that stopcocks *6* and *9* are not turned, so that flask *1* is connected to system during refluxing of sample in flask *2*. After sample has refluxed 20 min. close stopcocks *6* and *8* and then stopcock *11*, and note exact time of closing latter. Thus de-emanated sample is sealed in flask *2* by stopcocks *8* and *11*. Then detach this portion of app. at joints *7* and *10*, and attach another empty sample flask and condenser unit at joints *7* and *10*.

(5) *Radon accumulation.*—Let both std and sample stand sealed 2–30 days. As may be seen in **43.028**, ca 0.3 of equilibrium quantity of Rn is formed in 2 days, 0.5 in 4 days, and practically complete equilibrium is reached in 30 days. Sensitivity of detn is therefore controlled to large degree by period of storage of sample.

(6) *Background or natural leak.*—Connect electroscope and electrode with charging device so that negative potential of ca 300 volts (or potential necessary to set leaf or fibers to max. scale reading) is maintained on electrode and scope. Flush system by passing gentle stream of N thru empty sample flask *2*, the system, and ionization chamber 30 min. Then close stopcock *8* and evacuate system to 1 mm pressure. Close stopcocks *18* and *23*, shut off pump, and fill ionization chamber with N at atmospheric pressure. Make final adjustment of pressure with manometer *20*.

Remove source of negative potential from electrode and note time that leaf or fibers pass nearest division. Note exact time necessary for leaf or fibers to travel across one division and then let system stand until leaf or fibers travel across major portion of scale, noting time necessary. To obtain natural leak, calc. rate of discharge of instrument in "divisions/min."

(7) *Radon in std.*—Immediately after making final reading for natural leak, det. Rn content of std by transferring accumulated Rn in std soln from flask *1* to ionization chamber as follows:

Evacuate ionization chamber and app. to stopcock *9*, taking care that manometer *20* is shut off from system. Charge electroscope and electrode negatively and during refluxing maintain electrode at max. negative potential, as indicated on electroscope. When system is evacuated to ca 1 mm of Hg, as indicated on manometer *19*, manipulate stopcocks *6* and *9* very cautiously so that the Rn is carried in slow stream of N thru Hg bubble trap into evacuated chamber. Bring soln to boil and so adjust rate of N bubbling thru that at end of 20 min. refluxing, pressure within ionization chamber is slightly less than atmospheric pressure, as indicated on manometer *20*. (Gas velocity of Rn-N mixt. during refluxing is 2–4 bubbles/sec. as indicated in Hg bubble trap, and N flow is 1–2 bubbles/sec. as shown in flask.) After 20 min. refluxing, remove heat under flask *1* while N is still bubbling thru std soln. As soon as pressure in ionization chamber equals that of atmosphere, shown on manometer *20*, close stopcock *6* and then *9* to seal this part of system off again to allow another accumulation of Rn for future stdzns, and note exact time of closing stopcock *9*.

Let mixt. of Rn and N in ionization chamber stand 3 hr to let Rn form equilibrium quantities of Ra-A, -B and -C before taking readings. Re-

move source of negative potential from electrode and electroscope exactly 3 hr after stopcock *9* is closed, and take readings of electroscope with aid of stop-watch over same range as was used for natural leak detn.

After completing readings, pump out ionization chamber and rinse 2 or 3 times with pure N by evacuating and refilling ionization chamber, passing the N thru empty sample flask *2*.

(8) *Redetermination of natural leak.*—Let system stand at least 3 hr and redet. background of electroscope as directed previously.

(9) *Radon in sample.*—Disconnect empty sample flask *2* at joints *7* and *10* and replace with sealed flask *2*, which contains sample, after lubricating joint *10* with P_2O_5. For transfer of Rn from sample to ionization chamber and measurement of ionization, proceed as in (7).

(10) *Calculations.*—Subtract natural leak of electroscope in terms of divisions/min. from rate of fall in divisions/min. when Rn from std soln was in chamber. For sample sealed for <30 days calc. Rn content of std from **43.028.** For sample sealed 30 days or more, Rn content is substantially equiv. to Ra content. Divide calcd quantity of Rn by acceleration of rate of discharge of electroscope due to this quantity of Rn. Quotient is millimicrograms Ra equiv. to acceleration of 1 division/min. in rate of discharge of electroscope.

Subtract natural leak of electroscope in divisions/min. from accelerated rate of fall due to Rn of sample. Multiply this difference, which is net effect of Rn alone in sample, by millimicrograms Ra that cause increase of 1 division/min. as found in stdzn. If sample has been allowed to stand 30 days, result is quantity of Ra in subsample taken for analysis. If sample has stood <30 days, calc. Ra content from **43.028.** Report result in millimicrograms Ra/ml or g.

Gamma Ray Method Using Electroscope (2)—Official

(Applicable only to Ra in quantities $>10^{-5}$ g. This limit is arbitrary and depends on particular equipment used and accuracy required.)

40.006 APPARATUS

See Fig. 90. Cylindrical metal chamber *1*, ca 1 L capacity, is hermetically sealed. Axis of cylinder is vertical. On inside is Wulf 2-fiber system, fastened to amber insulator *4*, which can be charged with aid of electronic rectifier charging device or suitable charging rod. Rate of movement of fibers is detd by means of microscope *2*.

40.007 PREPARATION OF SAMPLE

(a) *Effective radioactivity.*—In case of devices and prepns in which radioactive material is not ingested, but applied externally, det. effective radioactivity directly upon sample without removing from container in which it is to be used.

(b) *Total radioactivity.*—Hermetically seal entire sample or one or more subdivisions in suitable container, such as test tube or flask. Let stand at least 30 days.

40.008 STANDARDS

Use known quantities of Ra measured by NBS.

40.009 STANDARDIZATION OF ELECTROSCOPE

Natural leak.—Charge electroscope thru charging rod by means of charging device to bring fibers to suitable point, for example at 70° division

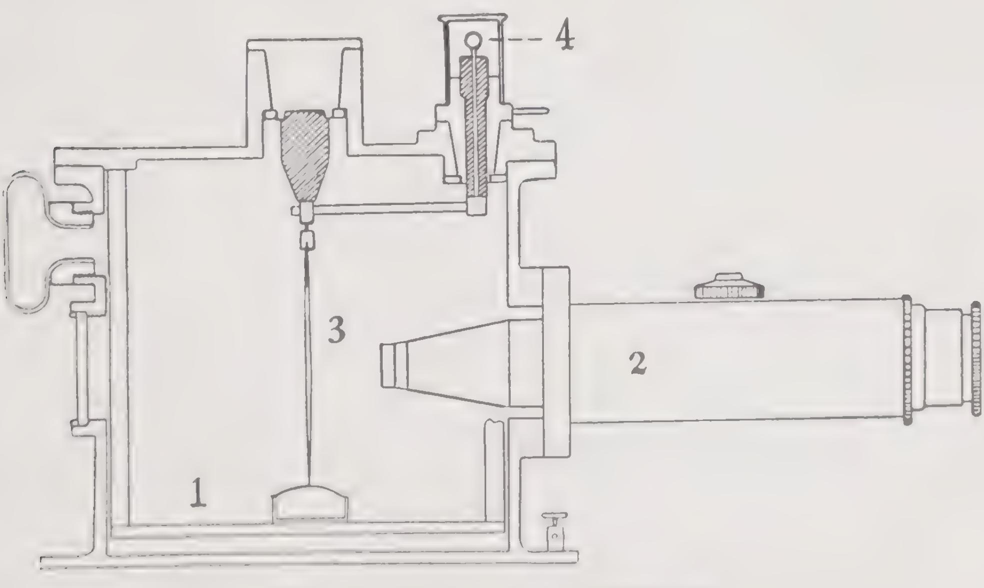

FIG. 90.—GAMMA RAY ELECTROSCOPE

mark, after charging rod *4* is grounded. As natural leak of electroscope in room free from Ra is very small, use Ra std to adjust fiber approximately to desired division mark. Remove std from room and record time when fiber crosses exact division mark. Let electroscope remain charged overnight. Again record time when one of fibers crosses exact division mark. Calc. rate of travel of fiber in sec./division and designate figure obtained as natural leak (R) of electroscope for particular detn.

40.010 DETERMINATION

(**a**) *Symbols.*—P = observed time of travel of fiber in sec./division due to std and natural leak; P' = observed time of travel of fiber in sec./division due to sample and natural leak; R = natural leak of electroscope in sec./division (*see* **40.009**); S = mmg Ra in std; S' = mmg Ra in sample; T = corrected time of travel in sec./division due to radioactivity of std alone; and T' = corrected time of travel in sec./division due to radioactivity of sample alone.

(**b**) *Measurement of sample.*—Place sample at such distance from electroscope as to give reasonable rate of travel of fiber. (Samples of very low radioactivity may be secured by means of rubber bands to circumference of electroscope so as to obtain max. ionization.) Charge electroscope as in **40.009** (Ra std may be used to adjust fiber). Record time, measured by stop-watch, for fiber to travel over that part of scale used in obtaining natural leak. Take total of 2 readings and calc. av. time of travel of fiber in sec./division (P'). Calc. corrected time of travel in sec./division (T') due to sample alone by formula: $T' = RP'/(R-P')$.

(**c**) *Measurement of std.*—Place suitable Ra std contg 10–1000 mmg Ra in exactly same position as sample was placed in (**b**) and charge electroscope as directed. Record time for fiber to travel over exact divisions used for sample. Take total of 2 readings and calc. av. time of travel of fiber in sec./division (P). Calc. corrected time of travel in sec./division (T) due to std alone by formula: $T = RP/(R-P)$.

(**d**) *Calculation of radioactivity of sample.*—Calc. mmg Ra (S') or equiv. radioactivity in terms of Ra by formula: $S' = ST/T'$.

Gamma Ray Method Using Geiger-Müller Counter (3)—Official

(Applicable only to Ra in quantities $>10^{-7}$ g. This limit is arbitrary and depends on particular equipment used and accuracy required.)

40.011 APPARATUS

Geiger-Müller discharge counter of coaxial type and appropriate electrical circuit for this counter. Response of instrument to gamma ray should be linear to at least 10,000 counts/min. and sensitivity such that 0.1 mmg Ra at least doubles background count.

40.012 STANDARDS

Set of 13 gamma-ray stds prepd and measured by NBS, ranging in value from 0.1 to 100 mmg Ra, in following steps: 0.1, 0.2, 0.2, 0.5, 1.0, 2.0, 2.0, 5.0, 10.0, 20.0, 20.0, 50.0, 100.

40.013 PREPARATION OF SAMPLE—
See **40.007**(**a**) and (**b**)

40.014 DETERMINATION

Turn instrument on and let stand at least 15 min. before detn is started.

1. Det. background count.
2. Place sample at such distance from counter that indication on meter may be conveniently read. Roughly estimating value of this reading, remove sample and put in its place Ra std of such strength that ca same reading is obtained.
3. Remove sample and std to where they do not affect background and wait 5 min. for background to become stable.
4. Make comparison of sample against std, placing each in same relative position to counter. Take check background readings after each pair of observations and allow enough time for instrument to come to equilibrium for each reading of sample, std, and background. This equilibrium time depends on time constant of instrument and may be from 1 to 5 min. or even longer. If individual counts are recorded, total count over each period is sufficient. If meter readings are recorded, make these readings at 30 sec. intervals during run and record av. Make 2 check detns.

Typical schedule:

	Time (min.)	Reading
1	0	Background run starts
	5	Background run ends
	Place sample in position	
2	10	Sample run starts
	15	Sample run ends
	Sample removed	
3	20	Background run starts
	25	Background run ends
	Place std in position	
4	30	Std run starts
	35	Std run ends
	Remove std	
5	40	Background run starts
	45	Background run ends

40.015 CALCULATION

1. Average each successive pair of background readings and subtract this av. background read-

ing from appropriate sample reading and std reading: *i.e.*, in example given above average 1 and 3 and subtract this av. from 2, and then average 3 and 5 and subtract from 4.

2. From the 3 comparisons 3 independent ratios of sample to std are obtained.

3. Calc. std deviation (S.D.) of measurement according to formula:

$$\text{S.D.} = \pm \sqrt{\frac{\Sigma(\bar{x} - x)^2}{n(n-1)}}.$$

where $\bar{x}$ = av. ratios of sample to std; x = each individual ratio of sample to std; n = number of ratios; and Σ = summation sign. (This S.D. computed from 3 measurements requires factor of 4.3 for conventional 95% confidence interval instead of usual 2.0 used when S.D. is obtained from large number of measurements.)

4. Multiply value of std by av. ratio and express result as mmg ($g \times 10^{-6}$) Ra ± S.D. (also in mmg Ra). This is radioactivity of subdivision used for analysis.

5. Express final result in millimicrograms ($g \times 10^{-9}$) or mmg ($g \times 10^{-6}$) Ra/g (or ml) ± S.D., multiplying or dividing "S.D." by same factor used for conversion of radioactivity of subdivision.

Radioactive Contamination—Procedure

Emergency Level Procedure (4)

40.016 APPARATUS

(a) *Portable count-rate meter.*—Consists of: (1) *Self-quenching glass Geiger-Müller tube*, side wall not >32 mg/sq. cm, mounted in slide opening metal shield; threshold approx. 800 volts, operated at ca midpoint of voltage plateau, slope of which is not >10%, connected with coaxial cable to (2) *Suitable power supply and electronic amplifier unit* with meter calibrated in milliroentgens (mr)/hr, connected thru sensitivity switch providing 3 ranges of scale reading, *e.g.*, 0–20, 0–2, and 0–0.2 mr/hr; linear response within each range.

(b) *Comparison std.*—Induces meter response identical to that from surface of H_2O contaminated with fission products decaying at rate of 2×10^5 disintegrations/min./ml (emergency tolerance level for H_2O to be consumed for not >10 day period). Construct such std as follows: Uniformly suspend suitable quantity "60-mesh" $UO_2(OAc)_2.2H_2O$ (ca 3 g, adjusted by trial) in 5 g liquid casting plastic, level, and solidify in shallow container, such as lid of ointment tin, ca 80 mm diam. and side wall 15 mm deeper than layer of plastic. Base of ointment tin, fitted with indented ring 15 mm below its edge, serves as container for liquids and finely divided solids to be tested, and to protect comparison std when not in use. Supplementary std of ½ this activity may be prepd similarly for monitoring supplies to be consumed over 30 day period.

40.017 DETERMINATION

With selectivity switch set for highest range (*e.g.*, 0–20 mr/hr), and with shield open, place G-M tube diametrically across std in contact with edge of container at 2 points. Adjust meter pointer to convenient value ca midway of scale with calibration screw and record reading as av. of fluctuations over 1–2 min. Duplicate reading should check within ±5%. Avoid extraneous radiation, such as that from luminous dial watch.

Fill sample container with liquid or finely divided solid to level of indented ring and obtain duplicate readings. Sample readings within ±100% of std reading are of practical quantitative significance for monitoring under emergency conditions.

SELECTED REFERENCES

(1) Rev. Sci. Instruments **4**, 216(1933); **6**, 99 (1935); Phys. Rev. **55**, 931(1939).
(2) J. Assoc. Offic. Agr. Chemists **19**, 101(1936).
(3) Ibid. **25**, 103, 618(1942).
(4) Ibid. **38**, 678(1955).

41. Spectroscopic Methods

EMISSION SPECTROGRAPHIC METHODS

Aluminum, Boron, Calcium, Copper, Iron, Magnesium, Manganese, Phosphorus, Potassium, Sodium, and Zinc in Plants (*1*)—First Action

41.001 PRINCIPLES

Methods described cover detn of major elements and common minor elements in plants, where spectrographic methods are valuable because of considerable number of elements that may be detd simultaneously. However, basic instrumental technics described are not limited to plant analysis. With appropriate modifications of sample prepn and details of stds, both methods may be adapted to many analytical problems involving other agricultural and biological materials.

Descriptions illustrate wide latitude permissible between methods capable of satisfactory performance. Two entirely different instrumental techniques are described which may be combined with any of the photometric methods. Points in both methods may be helpful and suggestive in instance where available equipment does not permit complete adherence to details of either method. "GENERAL RECOMMENDATIONS," **41.002**, are intended to be supplementarily helpful in such cases.

Direct current arc excitation method involves comparison of samples and stds of closely similar composition. Spectra are prepd by direct current arc excitation under chemically buffered conditions. Spark excitation method employs internal std technic, spark excitation, and correction system for matrix differences. It permits some leeway in composition between sample and std, and is advantageous for batches of samples of varying composition.

Substitutions in app. for either method may be made if based on approx. equiv. performance.

41.002 GENERAL RECOMMENDATIONS

(**a**) *Instrumental technic.*—If, because of equipment limitations, neither of described methods can be followed in detail, or if particular analytical problem involves detn of other elements, following procedure is recommended: Det. experimentally, to limits of facilities available, potentials of various electrode prepns and excitation conditions with relation to element detectability and general concn requirements. If set of conditions shows promise, make preliminary check for replicability of line indices.

For detn of very minute amounts of some elements in plants, preliminary chemical sepn and concn may be necessary. (Satisfactory procedure using 8-hydroxyquinoline is described by Mitchell (*2*).) Trace element concentrate so obtained may be combined with suitable matrix, and subsequent treatment adapted to regular instrumental technic.

Selection of analysis lines on basis of desirable intensity and freedom from spectral interference by other elements is facilitated by prepg spectrum of each component element at av. concn level at which it occurs in material to be analyzed. Align spectra collectively for comparison, preferably by exposure on same film or plate.

(**b**) *Precision.*—Stdze all conditions of technic and det. reproducibility of results by making ca 20 successive exposures on sample of representative composition. For each element calc. std deviation of single exposure and divide by square root of number of individual exposures that will be averaged in practice to constitute one detn. From this estimate of std deviation of single detn, calc. coefficient of variation for each element. Following upper limits for precision error of spectrographic detns in analysis of plant material are suggested as being satisfactory in relation to other routine methods, or to practical requirements: Coefficients of variation for K, Ca, and P, 5.0%; Mg, Mn, Fe, Al, Na, and Cu, 10.0; B, 15.0. Where only semiquantitative results are required, an overall coefficient of variation of 30% can be obtained.

(**c**) *Accuracy.*—Precise technic is essential, but is by no means only factor involved in ultimate accuracy. Reliability and appropriateness of stds and judgment used in reference procedure are of utmost importance. Failure in any of these respects can result in serious calibration error for otherwise satisfactory method.

Carefully prep. synthetic stds from highest grade H_2O-free analyzed chemicals, collectively blanked for minor and trace elements. Preferably confirm values assigned natural stds by results of more than one laboratory.

Necessity for matrix similarity between stds and samples, or for closely controlled correction system for matrix differences, is stressed in methods and is again emphasized. Check correction

scales frequently against stds which closely match particular types of plant materials being analyzed.

Remember that precision error of technic applies to reference exposures as well as to samples. For this reason, base fiducial adjustments on as many reference exposures as may feasibly be included in each series of samples.

Direct Current Arc Excitation Method

41.003 APPARATUS

(a) *Self-maintaining d.c. discharge.*

(b) *Large Littrow prism spectrograph.*—Equipped with custom built device for magnetically rotating arc envelope.

(c) *Photographic plates.*—Eastman Kodak III-O or K33.

(d) *Facilities for controlled processing of photographic plates.*

(e) *Lathe.*—For machining electrodes.

(f) *Comparator microphotometer.*

(g) *Lower electrodes.*—Cut std grade $\frac{5}{16}''$ diam. spectrographic C rods into $\frac{7}{8}''$ segments. In one end of each segment drill tapered hole for mounting on pedestal for burning; in other end drill crater 7 mm diam. ×4 mm deep; machine wall thickness to 0.4 mm. Purify these electrodes by preburning with ca 2.5 mg buffer, **41.004**(a), until buffer is consumed.

(h) *Upper electrodes.*—Use high purity C rods, $\frac{1}{8}''$ diam.

41.004 REAGENTS

(a) *Buffer.*—Mix equal parts, by wt, of Li_2SO_4 and pure spectrographic graphite powder.

(b) *Std solns.*—Make exploratory analysis of particular batch of samples to be analyzed. On basis of this analysis, prep. stock mixt. from individual pure Cl solns of respective elements, adjusting concns in mixt. so that they are equiv. to 0.1 of highest concn of each element in samples. Adjust acidity to 1 ml HCl/100 ml. Make successive dilns, each 40% or 50% of previous concn, to produce series of element concns covering indicated sample ranges. Use HCl (1+100) in prepg dilns. (It is important that major element levels be in ca same ratios to each other in these stds as they are in samples.)

(c) *Paraffin soln.*—Dissolve 12 g paraffin in enough kerosene to make 100 ml.

41.005 PREPARATION OF SAMPLES

Grind plant material in Wiley mill fitted with No. 40 sieve. Dry ground sample in oven and transfer accurately weighed 10 mg portions of dried sample to electrode craters, **41.003**(g). Insert charged electrodes in holes drilled in Transite block, and ash by placing block in cold muffle furnace and heating slowly to 500°. Remove from oven, and let cool. Waterproof electrodes contg ash by dropping 4 drops of the lukewarm paraffin soln, **41.004**(c), around each crater edge and letting air-dry. Add 1 drop H_2O and 2 drops HCl (1+30) to each crater to convert salts to chlorides. Dry treated electrodes at 100°.

Transfer accurately measured 0.1 ml portions of the std solns, **41.004**(b), to electrode craters which have been waterproofed with the paraffin soln, and dry in oven at 100°.

41.006 DETERMINATION

Add 4 mg of the buffer, **41.004**(a), to each of electrodes contg residues from samples and stds, **41.005**, align electrodes in holders, and burn to completion with current of 150 volts and 24 amperes. During burning period magnetically rotate gaseous envelope of arc to increase homogeneity, and maintain electrode alignment by periodic adjustment. Place stepped sector in light beam to permit simultaneous detn of Al, B, Ca, Cu, Fe, Mg, Mn, Na, and P. Replicate series of exposures 3 or 4 times on different plates.

Process photographic plates, **41.003**(c), under controlled conditions, as in **41.013.** Use following analysis lines: Ca, 2997.3; Mg, 2779.8; P, 2553.3; Mn, 2801.1 or 3054.4; Fe, 3020.6; Al, 3082.2; Na, 3302.3; Cu, 3247.5 or 3274.0; and B, 2496.8 or 2497.7 Å.

Det. element concn by photometry either semi-quantitatively, **40.016**(b), or quantitatively, **41.015** and **41.016**(a).

Alternating Current Spark Excitation Method

41.007 APPARATUS

(a) *Spark excitation source.*

(b) *Applied Research Laboratories 1.5 m grating spectrograph.*—With enclosed spark stand.

(c) *Photographic film.*—Eastman Kodak Spec. Anal. No. 1.

(d) *Film-processing equipment.*

(e) *Electrode drill.*

(f) *Calculating board and comparator microphotometer.*

(g) *Lower electrodes.*—Purify std grade spectrographic C rods, $\frac{1}{4}''$ diam., by succesive hot digestions with HCl (1+1), HNO_3 (1+1), and H_2O. Dry in oven. Cut purified rods into 2″ lengths. In one end of each 2″ rod drill crater 5 mm diam. ×6 mm deep, and pack crater with portion of C removed in drilling.

(h) *Upper electrodes.*—Point appropriate lengths of purified rod, (g), in pencil sharpener equipped with pin-stop to produce $\frac{1}{16}''$ diam. flat tip.

41.008 REAGENTS

(a) *Hydrochloric acid-cobalt dissolving soln.* Dil. 20 ml 2% Co soln and 300 ml HCl to 2 L.

(b) *Element soln.*—Prep. stock soln of each element from pure salt. Following concns are convenient: K, 5.0% and 0.5%; Ca, 3.0% and 0.3%; Mg and P, 1.0% and 0.1%; Mn, Fe, Al, Zn, and Na, 0.5%, 0.05%, and 0.005%; Cu and B, 0.05%, 0.005%, and 0.0005% (5 ppm); and Co, 2.0%.

(c) *Stock mixt.*—Prep. by combining following quantities of the element solns and dilg to 500 ml.

ELEMENT	CONCN OF STOCK SOLN %	QUANTITY ML	EQUIV. TO SAMPLE PERCENTAGE OF
K	5.0	50	0.5
Ca	3.0	50	0.3
Mg	1.0	35	0.07
P	1.0	35	0.07
Na	0.5	20	0.02
Fe	0.5	5	0.005 (50 ppm)
Al	0.5	5	0.005 (50 ppm)
Zn	0.5	5	0.005 (50 ppm)
Mn	0.5	2	0.002 (20 ppm)
Cu	0.05	5	0.0005 (5 ppm)
B	0.05	5	0.0005 (5 ppm)

Relation between stds and samples in fourth column is based on Cl residue from 1 g sample dissolved in 5 ml of the HCl-Co dissolving soln, (a). For each ml of the stock mixt. evapd and so dissolved, resulting std corresponds to listed percentage of the respective element.

41.009 PREPARATION OF STANDARDS

(Procedure is designed to cover analysis of miscellaneous plant material of various composition. Reference stds used, therefore, are of general nature. If requirements are confined to analysis of specific reasonably uniform types of plant material, stdzn procedure may be simplified to satisfy only variations involved. In such cases, adjust suggested proportional composition and concns of stds to approx. match material to be analyzed. In practice, spectra of 10 samples and duplicates of 2 reference stds are recorded on one film, and series of exposures is duplicated on another film. Averages of ratio values for the two films should be used in all cases.)

(a) *Stds for K, Mg, Fe, Al, Zn, Mn, Cu, and B curves.*—Prep. series of stds by evapg 1, 2, 4, 7, and 10 ml aliquots of the stock mixt., **41.008**(c), with 2 ml HCl and dissolving each residue in 5 ml of the HCl-Co dissolving soln, **41.008**(a). (P and Ca are affected by progressive increase in concn of matrix elements in these stds.)

(b) *Stds for P curve.*—Make 5 mixts from the element solns, **41.008**(b), each contg sample equivs of 2.5% K, 1.5% Ca, and 0.3% Mg. Add to these mixts 1, 2, 4, 6, and 8 ml, resp., of the 0.1% P soln. Add 2 ml HCl to each mixt., evap., and dissolve in 5 ml of the HCl-Co dissolving soln as in (a). (Because different K and Ca levels affect fiducial point rather than slope of P curve, 0.4% P present in all Ca stds serves to indicate relative fiducial adjustments necessary for various quantities of K and Ca present.)

(c) *Stds for Ca curve.*—To mixts, each contg sample equivs of 1.0% K, 0.3% Mg, and 0.4% P, add 1, 2, 4, 8, and 12 ml, resp., of the 0.3% Ca soln. Evap. and dissolve as in (b). (These stds represent 0.3% 0.6%, etc., of Ca, and are used to obtain Ca curve for K level of 1%.) Prep. 3 more sets of Ca stds by same procedure, raising K levels in these sets to 2.0%, 3.5%, and 5.0%, resp.

(d) *Stds for routine reference.*—Prep. low, medium, and high stds, from 2.5, 5, and 10 ml portions of the stock mixt., **41.008**(c), treated as in (a). (Addnl stds to match composition of unusual samples may easily be prepd from the element solns, **41.008**(b).)

41.010 PREPARATION OF SAMPLES

Weigh quantity of dry, ground material that will produce ash weighing preferably between 0.06 and 0.10 g. (1 g av. plant material meets this requirement.) Ash at 550°. Digest ash with excess HCl (1+1) and evap. to dryness on hot plate. Treat residue with 5 ml of the HCl-Co dissolving soln, **41.008**(a), and warm until salts dissolve. If any insol. material remains, let it settle out or remove by filtering thru small, dry filter.

Add, drop by drop, ca 0.1 ml sample or std soln to packed electrode, **41.007**(g), and let sink in. Dry treated electrodes 2 hr at 130° and keep in desiccator until sparked. (Rapidity with which soln soaks into electrode influences general density of spectrum. Individual electrodes, although prepd as nearly identically as possible, may vary considerably in this respect. Where absorption is very rapid, add addnl drop soln, so that some will remain on top of electrode several min. before being absorbed. If surface of electrode is glazed in packing operation, no soln will penetrate; in this case discard electrode.)

41.011 DETERMINATION

(a) *Excitation.*—Align and space electrodes ⅛" apart in holders with jig, and spark 25 sec. (This period is usually enough to empty crater; disregard any remaining material.) Set source parameters to give either uniform breakdown voltage at tandem air gap or to have the voltage at analytical gap in damped region for Applied Research Laboratories type source. Use 40 μ slit width and aperture which permits 14 exposures and clear strip for densitometer settings on film. Adjust gates so that total background, in general, is slight. Use no lens or filter. Make no background correction.

(b) *Emulsion calibration.*—Prep. emulsion calibration curves in 3000 and 4000 Å regions as in **41.015**(a).

(c) *Film processing.*—Process photographic

film, **41.007(c)**, under controlled conditions as in **41.013**.

(**d**) *Photometry*.—Use following analysis lines: K, 4044 or 4047; Ca, 2997; Mg, 2781 or 3337; P, 2553 or 2555; Mn, 2949, 2933, or 3460; Fe, 3020.6 or 3021.1; Al, 3082; Zn, 3345; Na, 3302; Cu, 3274; B, 2498; and Co (reference line), 3044 Å.

Det. log intensity ratios, **40.016(a)**, for all elements except K from 3000 Å emulsion calibration curve and those for K from 4000 Å emulsion calibration curve.

(While spectral density level equiv. to 25–30% *T* for Co reference line is desirable from standpoint of uniform element range coverage, density range between 20% and 50% *T* for this line does not affect validity of element/Co ratios. For this reason, density differences encountered between duplicate exposures provide excellent addnl check on emulsion calibration.)

Plot respective ratios against known percentages of elements in stds and draw analysis curves. Ratios from sample spectra may be referred directly to these curves, if desired, to obtain analysis values. Due to considerable number of curves involved in analysis of miscellaneous plant materials, however, it is more convenient to arrange data obtained from these curves as percentage scales for each element.

Mount blank cardboard strip parallel to horizontal logarithmic scale on calculating board, which is allowed to represent log I ratios. Mark complete range of percentage points on this strip in alignment with corresponding ratios on logarithmic scale. Fiducial relationship is adjustable by sliding strip in its mounting.

On the Ca strip, place the several scales corresponding to different level of K. On the P strip, place series of replicas of the P scale in fiducial alignment relative to different levels of combined K and Ca (*3*).

Correlate all analysis strips with the low, medium, and high general stds and mark percentage levels of these stds on the strips as fiducial reference points. Make fiducial adjustment for each set of duplicate films by aligning these reference points with resp. av. ratios for stds thereon. Refer accompanying samples to these settings.

In practice, determine K first. With K percentage known, proper scale to be used for Ca is indicated. With both K and Ca contents known, correct scale for P is indicated. Make no correction for matrix variation for other elements.

Photographic Processing

41.012 APPARATUS AND REAGENTS

(**a**) *Equipment*.—Developing machine; washer; dryer; large, easily read elec. timer; safelight; storage refrigerator; and water cooler.

(**b**) *Developer*.—Eastman Kodak D-19 or D-8.

(**c**) *Stop bath*.—2% HOAc.

(**d**) *Fixer*.—Eastman Kodak F-5 or Eastman rapid liquid.

41.013 TECHNIC

Store films or plates in refrigerator and remove 24 hr before opening to equilibrate to laboratory atmosphere. Use safelights appropriate to emulsion being processed. Carefully control time of each operation.

(**a**) *Developing*.—Place film or plate in developing machine contg developer and agitate 3–5 min. at constant temp. of 18–21°. Maintain same time and temp. for all films or plates of particular series. Immerse in stop bath of 2% HOAc for 30 sec. immediately after development.

(**b**) *Fixing*.—After plate or film is cleared in fixer, **41.012(d)**, fix at least 1 min. (longer for indefinite preservation of negative). Wash 3 min. in running H_2O at 18–21°, rinse in distd H_2O, and dry in 32° air in dryer.

Photometry

41.014 APPARATUS

(**a**) *Microphotometer.*

(**b**) *Seidel scale and calculating board.*

(**c**) *All-iron globule arc.*

(**d**) *Step sector or step filter.*

41.015 EMULSION CALIBRATION CURVES

Select emulsion with finest grain that line sensitivity will permit. Use all-Fe globule arc, run at very low amperage as light source. Align spectrograph optics to give max. uniformity of line intensity and check for synchronism and scattered light within spectrograph. Adjust microphotometer to focus sharply, with min. transmittance for given line. Set scanning element and spectrogram line parallel. Set microphotometer scale at 100% transmittance on clear area and zero transmittance for complete blackening. Check scale range frequently, and at least each time wavelength of spectrogram is changed. Det. response of emulsion to light by 2-step, step sector, 2 line, or line group method. Each method uses sep. system to measure variable quantities of light falling on emulsion. Step sector and 2-step method vary quantity of light in each line of spectrogram, whereas line and line group method rely on constant intensity differences between suitable lines of spectrum.

(**a**) *Line group method*.—Make spectrogram, using all-Fe globule arc as light source and power source, spectrograph, and microphotometer that will be used in analytical work. Use no sector or filter. Measure transmittance of selected lines in microphotometer and transform readings to Seidel function, **41.014(b)**. Plot Seidel functions against known relative intensities of selected lines to obtain emulsion calibration curve. Intensities are

listed in (*4*), but since observed, relative intensities are affected by source conditions and instrumental characteristics, check their scale validities by 2-step or step sector method. Where emulsion response varies with wavelength, draw calibration curves for each wavelength region used.

(**b**) *Two step method.*—Make spectrogram, using all-Fe globule arc as light source and power source, spectrograph, and microphotometer that will be used in analytical work. Use 2 step sector or step filter to give 2-step spectrogram. Measure transmittance of group of closely neighboring lines and transform to Seidel function, **41.014**(**b**). Prep. preliminary curve by plotting weak step of a line against strong step of same line for each line measured. Prep. preliminary curve table of values for weak and strong line steps, beginning at high point strong line value and reading off values for weak line as next value for strong line (*4*). Plot emulsion calibration curve, using this table and intervals equal to log of step ratio of sector or filter used. Make sep. emulsion calibration curves for each wavelength region used.

41.016 ANALYTICAL CURVE

Analytical curve is plot of log of concn of element in std versus either log intensity ratio or transmittance of line from that std. Intensity ratio curve is corrected for plate response and excitation variation and gives more precise results than transmittance curve. Latter gives adequate semiquant. or comparative results.

(**a**) *Intensity ratio curve.*—Prep. std spectrograms contg increasing quantities of each of elements to be analyzed and same quantity of internal std. Measure transmittance of lines, using only values in straight line portion of calibration curve, and transform to Seidel values. From calibration curve, **41.015**, read intensity value corresponding to Seidel value. Calc. intensity ratio of line of variable constituent to line of internal std. Plot log of intensity ratio against log of known concn. Where necessary, correct for background (*4*). Use calcg board, **41.014**(**b**) to perform mechanical operations of obtaining analytical curve.

(**b**) *Transmittance curve.*—Prep. std spectrograms as in **41.016**(**a**), measure transmittance of each line, and obtain quadruplicate values for each std. Use no internal std. Plot log of mean value against log of std concn. Run each replicate on different film or plate on different days. Treat samples for analysis in like manner.

SELECTED REFERENCES

(*1*) J. Assoc. Offic. Agr. Chemists **36**, 411 (1953); **37**, 721(1954).
(*2*) Mitchell, "The Spectrographic Analysis of Soils, Plants, and Related Materials," 1948.
(*3*) Anal. Chem. **25**, 946(1953).
(*4*) ASTM, "Methods for Emission Spectrographic Analysis," 2nd Ed., 1957.

42. Standard Solutions

42.001 General Directions (*1*)

Accurately calibrated equipment, which meets NBS specifications, should be used. Because alk. and other corrosive solns dissolve glass, to avoid vol. errors such solns should not be stored in calibrated app., and burets used continuously should be recalibrated periodically.

Working temp. of the std soln should approximate that of its temp. during stdzn. If temp. corrections are necessary, sufficient accuracy may be obtained by use of following table:

VOLUME OF STANDARD SOLN	CORRECTION IN MILLILITERS AT—												
	6°	8°	10°	12°	14°	16°	18°	20°	22°	24°	26°	28°	30°
ml													
10	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.00	0.00	0.00	−0.01	−0.02	−0.02
20	0.03	0.03	0.03	0.02	0.02	0.01	0.01	0.00	−0.01	−0.02	−0.03	−0.03	−0.03
25	0.04	0.03	0.03	0.03	0.02	0.02	0.01	0.00	−0.01	−0.02	−0.03	−0.04	−0.05
30	0.04	0.04	0.04	0.03	0.03	0.02	0.01	0.00	−0.01	−0.02	−0.04	−0.05	−0.07
40	0.06	0.06	0.05	0.04	0.04	0.03	0.01	0.00	−0.02	−0.03	−0.05	−0.07	−0.09
50	0.07	0.07	0.06	0.06	0.05	0.03	0.02	0.00	−0.02	−0.04	−0.06	−0.09	−0.12

Ammonium and Potassium Thiocyanates (*2*)—Official

42.002 REAGENTS

(a) *Purified silver nitrate.*—Dissolve 50 g $AgNO_3$ in 20 ml boiling H_2O contg ca 5 drops HNO_3. Heat to dissolve, filter while still hot thru fritted glass filter, using suction, and collect filtrate in clean Pyrex beaker. Wash beaker and filter with ca 5 ml hot H_2O, adding washings to filtrate. Cool in ice bath, stirring to induce crystn, and place in refrigerator at ca 10° until equilibrium is reached. Decant liquid thru fritted glass filter and transfer crystals to filter. Cover filter with watch glass and draw air thru filter to remove adhering liquid. Transfer crystals to small, clean Pyrex beaker. Cover beaker with watch glass and place inside larger covered Pyrex beaker. Dry at 105° and fuse at 220–250° (m.p. 208°), holding at this temp. ca 15 min. after crystals are melted. Protect from dust during prepn. Cool in desiccator, remove product from beaker, powder in mortar, dry 0.5 hr at 105°, and store in brown g-s. bottle in dark over good desiccant.

(b) *Reference soln.*—To mixt. of 5 ml HNO_3 (1+1), 2 ml Fe alum soln, **42.028(a)**, and 115 ml H_2O, add ca 0.02 ml 0.1*N* thiocyanate, noting exact quantity used.

42.003 PREPARATION OF STANDARD SOLUTION—*See* 42.028 (b)

42.004 STANDARDIZATION

Weigh accurately, on tared watch glass, enough purified $AgNO_3$ to give titrn of ca 40 ml (ca 0.7 g for 0.1*N* soln) and transfer with H_2O thru glass funnel to 250 ml g-s. erlenmeyer. Dissolve in ca 75 ml H_2O (halogen-free), and add 5 ml HNO_3 (1+1) and 2 ml Fe alum soln, **42.028(a)**. Titr. with the thiocyanate soln to appearance of reddish-brown color, which remains after shaking vigorously 1 min. Record buret reading and set flask aside 5 min., shaking occasionally and maintaining end point color by addn of thiocyanate soln as required. Then add addnl thiocyanate soln, if necessary, to produce permanent end point color, matching with color of reference soln, **42.002(b)**. From total vol. thiocyanate soln used in titrn subtract quantity contained in reference soln.

$$\text{Normality} = \frac{\text{g } AgNO_3 \times 1000}{\text{ml titer} \times 169.89}.$$

Arsenious Oxide (*3*)—Official

42.005 REAGENT

Arsenious oxide.—Use NBS Standard Sample. Dry 1 hr at 105° immediately before using.

42.006 PREPARATION OF STANDARD SOLUTION

Weigh As_2O_3 accurately by difference from small g-s. weighing bottle (use ca 4.95 g/L for 0.1*N*). Dissolve in 1*N* NaOH (50 ml/5 g As_2O_3) in flask or beaker by heating on steam bath. Add ca same quantity of 1*N* H_2SO_4. Cool, transfer mixt. quantitatively to vol. flask, and dil. to vol. (Soln must be neutral to litmus, not alk.)

Buffer Solutions for Calibration of pH Equipment (*4*)—Official

42.007 PREPARATION OF STANDARD BUFFER SOLUTIONS

Use H_2O with pH of not <6.5 nor >7.5 obtained by boiling H_2O 15 min. and cooling under CO_2-free conditions. Store std buffer solns in bottles of chemically resistant glass.

(a) *Potassium tetroxalate buffer soln.*—0.05*M*. Transfer 12.70 g $KHC_2O_4.H_2C_2O_4.2H_2O$ (air wt) (NBS Standard Sample 189) to 1 L vol. flask, dil. to mark with H_2O, and mix thoroly. (It is not necessary to remove dissolved CO_2 from the H_2O or to dry salt before weighing.) Prep. fresh every 2 months.

(b) *Potassium hydrogen tartrate buffer soln.*—Satd soln at 25°. Add excess (ca 100%) of $KHC_4H_4O_6$ (NBS Standard Sample 188) to H_2O in g-s. bottle or flask, and shake vigorously; few min. shaking is enough for satn (100 ml H_2O at 25° dissolves ca 0.7 g $KHC_4H_4O_6$). Adjust to 25°, let solid settle, and decant clear soln, or filter if necessary. Discard when mold appears. Few crystals of thymol added during prepn will retard mold growth, and will alter pH by <0.01 unit. For accuracy of ±0.01 pH unit, temp. of soln at satn must be between 20° and 30°.

(c) *Acid potassium phthalate buffer soln.*—0.05*M*. Dissolve 10.211 g dried (1 hr at 105°) $HKC_8H_4O_4$ (NBS Standard Sample 185) in H_2O and dil. to 1 L. (Elaborate precautions for exclusion of atmospheric CO_2 are unnecessary, altho soln should be protected against evapn and contamination with molds. Replace soln if mold appears.

(d) *Phosphate buffer soln.*—0.025*M*. Dissolve 3.402 g KH_2PO_4 and 3.549 g Na_2HPO_4 (NBS Standard Samples 186-I and II) in H_2O and dil. to 1 L. (Dry salts 2 hr at 130° before use; no special precautions to prevent contamination of the buffer soln with atmospheric CO_2 are necessary.)

(e) *Borax buffer soln.*—0.01*M*. Dissolve 3.814 g $Na_2B_4O_7.10H_2O$ (NBS Standard Sample 187) in H_2O and dil. to 1 L. (Salt must not be dried in oven before use.) To avoid contamination with CO_2, stopper bottle except when in use or protect with soda-lime tube. Use buffer soln within 10 min. after removal from bottle.

42.008 PH VALUES FOR STANDARD BUFFER SOLUTIONS

pH values of std buffer solns as function of temp. are given in following table:

TEMPERATURE	0.05*M* POTASSIUM TEXTROXALATE	SATD POTASSIUM HYDROGEN TARTRATE	0.05*M* ACID POTASSIUM PHTHALATE	0.025*M* PHOSPHATE	0.01*M* BORAX
°*C*	*pH*	*pH*	*pH*	*pH*	*pH*
0	1.67	—	4.01	6.98	9.46
5	1.67	—	4.01	6.95	9.38
10	1.67	—	4.00	6.92	9.33
15	1.67	—	4.00	6.90	9.27
20	1.68	—	4.00	6.88	9.22
25	1.68	3.56	4.01	6.86	9.18
30	1.69	3.55	4.01	6.85	9.14
35	1.69	3.55	4.02	6.84	9.10
40	1.70	3.54	4.03	6.83	9.07
45	1.70	3.55	4.04	6.83	9.04
50	1.71	3.55	4.06	6.83	9.01
55	1.72	3.56	4.08	6.83	8.99
60	1.73	3.57	4.10	6.83	8.96

Hydrochloric Acid

42.009 PREPARATION OF STANDARD SOLUTIONS

Following table gives approx. quantities of HCl (reagent quality, 35–37% HCl) required to make 10 L std solns:

Approx. normality	*ml HCl to be dild to 10 L*
0.01	8.9
0.02	17.8
0.10	89.0
0.50	445.0
1.0	890.0

42.010 *Standard Sodium Hydroxide Method (5)—Official*

Titr. 40 ml against std alkali soln of ca same concn as acid being stdzd as in **42.033**, using phthln.

Normality

= (ml std alkali × normality of alkali)/ml HCl.

If more concd than desired, dil. soln to required normality value by following formula:

$$V_1 = V_2 \times N_2/N_1,$$

where N_2 and V_2 represent normality and vol. of stock soln, resp., and V_1 represents vol. to which stock soln should be dild to obtain desired normality, N_1.

Check exact concn of final soln by titrn as above. Normality will be exact only if same indicator is used in detn as in stdzn.

If the std acid soln is to be used with Me orange as indicator, det. correction for vol. of acid required to pass from end point of phthln to that of Me orange. Add 3 drops 1% alc. phthln soln to 100 ml CO_2-free H_2O, and then add enough alkali soln to give end point with phthln. Disregard quantity of alkali soln added and take buret readings from this point. Add 3 drops 0.02% Me orange and enough 0.1*N* acid to produce pink color of Me orange. Titr. back with 0.1*N* alkali

soln to same end point taken in usual titrn (preferably pH 4.2).

Buffer solns useful in accurately detg Me orange end point are prepd (*6*) by dissolving 2.041 g KH phthalate in H_2O and adding 5.30 ml 0.1*N* HCl, 0.80 ml 0.1*N* NaOH, and 7.30 ml 0.1*N* NaOH, and dilg to 200 ml with H_2O for pH 3.8, 4.0, and 4.2, resp.

If acid and alkali solns are equiv., ml acid − ml alkali soln = ml acid required to pass from phthln end point to that of Me orange.

42.011 *Constant Boiling Method (7)—Official*

Dil. 850 ml HCl (35–37% HCl) with 750 ml H_2O. Check sp. gr. with spindle and adjust to 1.10. Place 1.5 L in 2 L flat-bottom distg flask, add ca 10 SiC grains (ca "20 mesh"), and connect to long, straight inner-tube condenser. Heat on elec. hot plate and distill at rate of 5–10 ml/min., keeping end of condenser open to air. When 1125 ml has distd, change receivers and catch next 225 ml, which is constant boiling HCl, in erlenmeyer with end of condenser inserted into flask, but above surface of liquid. Read barometer to nearest mm at beginning and end of collection of 225 ml portion and note barometer temp. Average readings.

Calc. air wt in g (*G*) of this constant boiling HCl required to give one equiv. wt of HCl from one of the following equations:

For P_0 = 540–669 mm Hg:

$$G = 162.255 + 0.02415\, P_0$$

For P_0 = 670–780 mm Hg:

$$G = 164.673 + 0.02039\, P_0$$

P_0 = barometric pressure in mm Hg corrected to 0°C for expansion of Hg and of barometer scale. For brass scale barometer, following correction is accurate enough: $P_0 = P_t(1-0.000162t)$, where t = barometer temp. in °C.

Weigh required quantity of constant boiling HCl in tared, stoppered flask with accuracy of at least one part in 10,000. Dil. immediately, and finally dil. to vol. with CO_2-free H_2O at desired temp.

Standard Borax Method (8)—Official

42.012 REAGENTS

(a) *Methyl red indicator.*—Dissolve 100 mg Me red in 60 ml alcohol and dil. with H_2O to 100 ml.

(b) *Reference soln.*—Prep. reference soln of H_3BO_3, NaCl, and indicator corresponding to composition and vol. of the soln at equivalence point. For use in detn of end point of titrn with 0.1*N* HCl, reference soln should be 0.1*M* in H_3BO_3 and 0.05*M* in NaCl.

(c) *Std borax.*—Sat. 300 ml H_2O at 55° (not higher) with $Na_2B_4O_7.10H_2O$ (ACS) (ca 45 g). Filter at this temp. thru folded paper into 500 ml erlenmeyer. Cool filtrate to ca 10°, with continuous agitation during crystn. Decant supernatant, rinse ppt once with 25 ml cold H_2O, and dissolve crystals in just enough H_2O at 55° to insure complete soln (ca 200 ml). Recrystallize by cooling to ca 10°, agitating flask during crystn.

Filter crystals onto small büchner with suction, wash ppt once with 25 ml ice-cold H_2O, and dry crystals (*9*) by washing with two 20 ml portions alcohol, drying after each washing with suction. Follow with two 20 ml portions ether. (Just before use, free alcohol and ether from any possible reacting acids by shaking each vigorously with 2–3 g of the pure, dry $Na_2B_4O_7.10H_2O$ and then filtering.) Spread crystals on watch glass, immediately place dried $Na_2B_4O_7.10H_2O$ in closed container over soln satd with respect to both sugar and NaCl, and let it remain at least 24 hr before using. Then transfer the pure $Na_2B_4O_7.10H_2O$ to g-s. container and store in closed container over soln satd with respect to both sugar and NaCl when not in use (stable under these conditions 1 year).

42.013 STANDARDIZATION

Accurately weigh enough std $Na_2B_4O_7.10H_2O$ to titr. ca 40 ml and transfer to 300 ml flask. Add 40 ml CO_2-free H_2O and stopper flask. Swirl gently until sample dissolves. Add 4 drops Me red and titr. with soln that is being stdzd to equivalence point as indicated by reference soln.

$$\text{Normality} = \frac{\text{g } Na_2B_4O_7.10H_2O \times 1000}{\text{ml acid} \times 190.72}.$$

Standard Sodium Carbonate Method (8)—Official

42.014 REAGENTS

(a) *Methyl orange indicator.*—0.1% in H_2O.

(b) *Reference soln.*—80 ml CO_2-free H_2O contg 3 or 4 drops Me orange.

(c) *Anhydrous sodium carbonate (10).*—Heat 250 ml H_2O to 80° and add $NaHCO_3$ (ACS), stirring until no more dissolves. Then filter soln thru folded paper (use of hot H_2O funnel is desirable) into erlenmeyer. Cool filtrate to ca 10°, swirling constantly during crystn. Fine crystals of trona that sep. out have approx. composition: $Na_2CO_3.NaHCO_3.2H_2O$. Decant mother liquor, drain crystals by suction, and wash once with cold H_2O.

Transfer ppt, being careful not to include any paper fibers, to large flat-bottom Pt dish. Heat 1 hr at 290° in elec. oven or furnace with pyrometer control. Stir contents occasionally with Pt wire. After heating, cool in desiccator. Place the anhyd. Na_2CO_3 in g-s. container and store in

desiccator contg efficient desiccant. Dry at 120° just before using.

42.015 STANDARDIZATION

Accurately weigh enough anhyd. Na_2CO_3 to titr. ca 40 ml, transfer to 300 ml erlenmeyer, and dissolve in 40 ml H_2O. Add 3 drops Me orange and titr. (*11*) until color begins to deviate from H_2O tint (reference soln). (Equivalence point has not been reached.) Boil soln gently 2 min.; then cool. Titr. until color is barely different from H_2O tint of indicator.

$$\text{Normality} = \frac{\text{g } Na_2CO_3 \times 1000}{\text{ml acid} \times 52.997}.$$

Iodine (*3*)—Official

42.016 PREPARATION OF STANDARD SOLUTION

Dissolve weighed quantities of I (12.7 g/L for 0.1*N* soln) and KI, in proportion of 20 g KI to 13 g I, in 50 ml H_2O. When I dissolves, transfer soln to g-s. vol. flask. Dil. to mark with H_2O and mix thoroly. Keep soln in dark brown g-s. bottle away from light and restdze as frequently as necessary.

42.017 STANDARDIZATION

Transfer accurately measured portion of std As_2O_3 soln, **42.006**, (40–50 ml ca 0.1*N* soln for 0.1*N* I soln) to erlenmeyer. Acidify slightly with H_2SO_4 (1+10), neutralize with solid $NaHCO_3$, and add ca 2 g excess. Titr. with the I soln, using ca 0.2% starch soln (5 ml/100 ml) as indicator. Sat. soln with CO_2 at end of titrn by adding 1 ml of the dil. H_2SO_4 just before end point is reached.

$$\text{Normality} = \frac{\text{ml } As_2O_3 \times \text{normality } As_2O_3}{\text{ml I}}.$$

Potassium Bromide-Bromate (*12*)—Official

42.018 PREPARATION OF STANDARD SOLUTION

Dissolve ca 2.8 g $KBrO_3$ and 12 g KBr in boiled H_2O and dil. to 1 L with boiled H_2O for ca 0.1*N* soln.

42.019 STANDARDIZATION

Measure 40 ml of the std As_2O_3 soln, **42.006**, from buret into 300 ml erlenmeyer. Add 10 ml HCl and 3 drops Me orange, **42.014(a)**. Titr. with the KBr-$KBrO_3$ soln until 1 drop, or less, causes color of the Me orange to fade completely. Swirl soln constantly and add last ml dropwise with swirling between drops.

$$\text{Normality} = \frac{\text{ml } As_2O_3 \times \text{normality } As_2O_3}{\text{ml KBr-}KBrO_3}.$$

Potassium Dichromate (*13*)—Official

42.020 REAGENT

Starch soln. Mix ca 1 g arrowroot starch with 10 ml H_2O and pour slowly, with constant stirring, into 200 ml boiling H_2O. Boil until thin, translucent fluid is obtained. Let settle and use clear supernatant. Preserve with Hg.

42.021 ASSAY OF STOCK POTASSIUM DICHROMATE

If the $K_2Cr_2O_7$ is in small crystals, composite it by shaking thoroly in large, clean jar; if it is in lumps, grind representative sample until it passes thru No. 60 sieve, and then composite by shaking. Dry portion for weighings 2 hr at 100°.

Weigh, into each of 3 g-s. erlenmeyers, enough NBS Standard Sample $K_2Cr_2O_7$ to give titer of 100.5–102.0 ml 0.1*N* $Na_2S_2O_3$, **42.035** (0.4929–0.5002 g for 0.1*N* soln). Completely dissolve in 100 ml H_2O, add 4.0 g KI, and swirl mixt. until dissolved. With buret, add 4.0 ml HCl, stopper flask, mix by swirling, and let stand in dark 10 min. Cool flask ca 1 min. in ice-H_2O.

While swirling flask, pipet in 100 ml of the $Na_2S_2O_3$ soln. Add 5 ml of the starch soln and complete titrn with the $Na_2S_2O_3$ soln added from 10 ml microburet (graduated in 0.05 ml). End point is from bluish-green to clear green; change takes place within 0.01 ml. Record titer to nearest 0.01 ml. Calc. apparent normality of the $Na_2S_2O_3$ soln for each of the 3 titrns, and average. Designate this av. as N_{NBS}.

Similarly titr. 3 portions of the stock $K_2Cr_2O_7$ and calc. the 3 apparent normalities. Designate each of these results as N_{stock}. Calc. % purity of the stock $K_2Cr_2O_7 = (N_{NBS} \times 100)/N_{stock}$.

Take av. of the 3 results as % purity of the stock $K_2Cr_2O_7$.

42.022 PREPARATION OF STANDARD SOLUTION

Dissolve theoretical quantity of NBS Standard Sample $K_2Cr_2O_7$ (4.9037 g for 0.1*N* soln), or quantity of the stock $K_2Cr_2O_7$, **42.021**, found to have oxidimetric value 99.95–100.05% of NBS Standard Sample, in enough H_2O to make 1 L. (Dry the $K_2Cr_2O_7$ 2 hr at 100° before weighing.)

Potassium Permanganate (*14*)—Official

42.023 PREPARATION OF STANDARD SOLUTION

Dissolve slightly more than desired equiv. wt (3.2 g for 0.1*N*) of $KMnO_4$ in 1 L H_2O. Boil soln 1 hr. Protect from dust and let stand overnight. Thoroly clean 15 cm glass funnel, perforated porcelain plate from Caldwell crucible, and g-s. bottle (preferably of brown glass) with warm H_2SO_4-$K_2Cr_2O_7$ soln. Digest asbestos for use in gooches on steam bath 1 hr, with ca 0.1*N* $KMnO_4$ that has been acidified with few drops H_2SO_4 (1+3). Let settle, decant, and replace with H_2O.

To prep. glass funnel, place porcelain plate in apex, make pad of asbestos ca 3 mm thick on plate, and wash acid-free. (Pad should not be too tightly packed and only moderate suction should be applied.) Insert stem of funnel into neck of bottle and filter the $KMnO_4$ soln directly into bottle without aid of suction.

42.024 STANDARDIZATION

Transfer 0.3 g dried (1 hr at 105°) NBS Standard Sample Na oxalate to 600 ml beaker. Add 250 ml H_2SO_4 (5+95) previously boiled 10–15 min. and then cooled to 27 ±3°.

Stir until the $Na_2C_2O_4$ dissolves. Add 39–40 ml of the $KMnO_4$ soln at rate of 25–35 ml/min., stirring slowly. Let stand until pink disappears (ca 45 sec.). If pink should persist because the $KMnO_4$ soln is too concd, discard, and begin again, adding few ml less of the $KMnO_4$ soln. Heat to 55–60°, and complete titrn by adding the $KMnO_4$ soln until faint pink persists 30 sec. Add last 0.5–1 ml dropwise with particular care to let each drop decolorize before adding next.

Det. excess of $KMnO_4$ soln required to impart pink color to the soln by matching with color obtained by adding $KMnO_4$ soln to same vol. of the boiled and cooled dil. H_2SO_4 at 55–60°. This correction is usually 0.03–0.05 ml. From net vol. $KMnO_4$, calc. normality:

$$N = \frac{\text{g } Na_2C_2O_4 \times 1000}{\text{ml } KMnO_4 \times 67.002}.$$

Silver Nitrate (*15*)—Official

42.025 PREPARATION OF STANDARD SOLUTION

Dissolve slightly more than theoretical quantity of $AgNO_3$ (equiv. wt, 169.89) in halogen-free H_2O and dil. to vol. Thoroly clean glassware, avoid contact with dust, and keep prepd soln in amber g-s. bottles away from light.

Mohr Method

42.026 REAGENTS

(a) *Potassium chloride.*—Recrystallize KCl 3 times with H_2O, dry at 110°, and then heat at ca 500° to constant wt. Equiv. wt KCl = 74.557.

(b) *Potassium chromate soln.*—5% soln of K_2CrO_4 in H_2O.

42.027 STANDARDIZATION

Weigh accurately enough KCl to yield titrn of ca 40 ml (ca 0.3 g for 0.1N soln), and transfer to 250 ml g-s. erlenmeyer with 40 ml H_2O. Add 1 ml of the K_2CrO_4 soln and titr. with the $AgNO_3$ soln until first perceptible pale red-brown color appears. Subtract from titrn vol. ml of the $AgNO_3$ soln required to produce end point color in 75 ml H_2O contg 1 ml of the K_2CrO_4 soln. From net vol. $AgNO_3$, calc. normality:

$$N = \frac{\text{g KCl} \times 1000}{\text{ml } AgNO_3 \times 74.557}.$$

Volhard Method

42.028 REAGENTS

(a) *Ferric alum indicator soln.*—Satd soln of $FeNH_4(SO_4)_2.12H_2O$ in H_2O.

(b) *Potassium or ammonium thiocyanate std soln.*—Prep. ca 0.1N soln from reagent that shows no Cl (7.613 g NH_4CNS or 9.719 g KCNS/L). Det. working titer by accurately measuring 40–50 ml of the std $AgNO_3$ soln, adding 2 ml of the Fe alum soln and 5 ml HNO_3 (1+1), and titrg with the thiocyanate soln until soln shows pale rose color after vigorous shaking.

42.029 STANDARDIZATION

Weigh accurately enough of the KCl, **42.026**(a), to yield titrn of ca 40 ml (ca 0.3 g for 0.1N soln) and transfer to 250 ml g-s. erlenmeyer with 40 ml H_2O. Add 5 ml HNO_3 (1+1) and add excess of the $AgNO_3$ soln. Mix, and let stand few min. protected from light. Filter thru gooch prepd with medium pad of asbestos previously rinsed with 2% HNO_3. Wash flask and ppt with several small portions of 2% HNO_3, passing washings thru crucible until filtrate and washings measure ca 150 ml. Add 2 ml of the Fe alum soln and titr. residual $AgNO_3$ with the thiocyanate soln. From titrn, together with ratio of the 2 solns, calc. net vol. $AgNO_3$ soln. (Errors of blank are compensating and may be disregarded.) From net vol. $AgNO_3$, calc. normality as in **42.027.**

Sodium Hydroxide

Standard Acid Potassium Phthalate Method (16)—Official

42.030 APPARATUS

Use buret and pipet calibrated by NBS or by analyst. Protect exits to air of automatic burets from CO_2 contamination by suitable guard tubes contg soda-lime. Use containers of alkali-resistant glass.

42.031 REAGENTS

(a) *Carbonate-free water.*—Prep. by one of following methods: (1) Boil H_2O 20 min. and cool with soda-lime protection; (2) bubble air, freed from CO_2 by passing thru tower of soda-lime, thru H_2O 12 hr.

(b) *Sodium hydroxide soln.*—(1+1). To 1 part NaOH (reagent quality contg <5% Na_2CO_3) in flask add 1 part H_2O and swirl until soln is complete. Close with rubber stopper. Set aside until Na_2CO_3 has settled, leaving perfectly clear liquid (ca 10 days).

(c) *Acid potassium phthalate.*—NBS Standard Sample for acidimetry. Dry 2 hr at 120°. Cool in desiccator contg H_2SO_4.

42.032 PREPARATION OF STANDARD SOLUTION

Following table gives approx. quantities of NaOH soln (1+1) necessary to make 10 L of std solns:

Approx. normality	*ml NaOH to be dild to 10 L*
0.01	5.4
0.02	10.8
0.10	54.0
0.50	270.0
1.0	540.0

Add required quantity of NaOH soln (1+1) to 10 L CO_2-free H_2O. Check normality, which should be slightly high, as in **42.033**, and adjust to desired concn by following formula: $V_1 = V_2 \times N_2/N_1$, where N_2 and V_2 represent normality and vol. stock soln, resp., and V_1, vol. to which stock soln should be dild to obtain desired normality, N_1. Stdze final soln as in **42.033**.

42.033 STANDARDIZATION

Accurately weigh enough dried $KHC_8H_4O_4$ to titr. ca 40 ml and transfer to 300 ml flask that has been swept free from CO_2. Add 50 ml cool CO_2-free H_2O. Stopper flask and swirl gently until sample dissolves. Add 3 drops phthln, and titr. with soln being stdzd.

$$\text{Normality} = \frac{\text{g } KHC_8H_4O_4 \times 1000}{\text{ml NaOH} \times 204.228}.$$

(Normality value is exact only when phthln is used as indicator.)

42.034 *Constant Boiling Hydrochloric Acid Method (17)—Official*

Accurately weigh from weighing buret enough constant boiling HCl, **42.011**, to titr. ca 40 ml, into erlenmeyer previously swept free from CO_2. Add ca 40 ml CO_2-free H_2O and then 3–5 drops of desired indicator, and titr. with soln being stdzd.

$$\text{Normality} = \frac{\text{g HCl} \times 1000}{\text{ml titer} \times G},$$

where G has value given in **42.011**.

Sodium Thiosulfate (*18*)—Official

42.035 PREPARATION OF STANDARD SOLUTION

Dissolve ca 25 g $Na_2S_2O_3.5H_2O$ in 1 L H_2O. Boil gently 5 min. and transfer while hot to storage bottle previously cleaned with hot H_2SO_4-$K_2Cr_2O_7$ soln and rinsed with warm boiled H_2O. (Temper bottle, if not made of resistant glass, before adding hot soln.) Store soln in dark, cool place; do not return unused portions to stock bottle. If solns less concd than $0.1N$ are desired, prep. by diln with boiled H_2O. (More dil. solns are less stable and should be prepd just before use.)

42.036 STANDARDIZATION

Accurately weigh 0.20–0.23 g $K_2Cr_2O_7$ (NBS Standard Sample dried 2 hr at 100°) and place in g-s. I flask (or g-s. flask). Dissolve in 80 ml Cl-free H_2O contg 2 g KI. Add, with swirling, 20 ml ca $1N$ HCl and immediately place in dark 10 min. Titr. with the $Na_2S_2O_3$ soln, **42.035**, adding starch soln after most of the I has been consumed.

$$\text{Normality} = \frac{\text{g } K_2Cr_2O_7 \times 1000}{\text{ml } Na_2S_2O_3 \times 49.037}.$$

Sulfuric Acid

42.037 PREPARATION OF STANDARD SOLUTION

Following table gives approx. quantities of H_2SO_4 (ca 94% H_2SO_4) necessary to make 10 L std solns:

Approx. normality	*ml H_2SO_4 to be dild to 10 L*
0.01	2.8
0.02	5.7
0.10	28.4
0.50	141.8
1.0	283.5

Standard Borax Method (19)—Official

42.038 STANDARDIZATION—*See* **42.013**

42.039 *Specific Gravity Method (20)—Official*

Dil. H_2SO_4 with enough H_2O to make convenient quantity of ca 70% H_2SO_4 by wt. Det. sp. gr. in air at convenient temp. (0–40°) as in **9.011** (or sp. gr. may be detd with Sprengel pycnometer), protecting soln from contact with air. Calc. exact % H_2SO_4 from equation: $P = S(85.87 + 0.05T - 0.0004t^2) - 69.82$, where P = % H_2SO_4 by wt and S = sp. gr. (in air) at T°, compared with H_2O at t°.

Weigh exactly W g prepd acid contg P% H_2SO_4 and dil. to n L to make required soln contg A g H_2SO_4/L. W may be calcd by equation: $W = nA \times 100/P$.

Titanium Trichloride (*21*)—Official

42.040 PREPARATION OF STANDARD SOLUTION

To 200 ml commercial 15% $TiCl_3$ soln add 150 ml HCl and dil. to 2 L. Make soln ca $0.1N$, place in container with H atmosphere provision (*22*), and let stand 2 days for absorption of residual O.

42.041 STANDARDIZATION

Weigh 3 g $FeSO_4(NH_4)_2SO_4.6H_2O$ and transfer to 500 ml flask. Introduce stream of CO_2 and add 50 ml recently boiled H_2O and 25 ml 40% (by wt) H_2SO_4. Then, without interrupting current of

CO_2, add rapidly 40 ml 0.1N $K_2Cr_2O_7$, **42.022**. Add the $TiCl_3$ soln until near calcd end point. Then add quickly 5 g NH_4CNS, and complete titrn. Det. blank on 3 g $FeSO_4(NH_4)_2SO_4.6H_2O$, using same quantities of H_2O, H_2SO_4, and NH_4CNS, and current of CO_2. From net vol. $TiCl_3$, calc. normality:

$$N = \frac{\text{ml } K_2Cr_2O_7 \times \text{normality } K_2Cr_2O_7}{\text{ml } TiCl_3}.$$

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(*3*) Ibid. **22**, 568(1939); **24**, 100, 639(1941).

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(*5*) J. Assoc. Offic. Agr. Chemists **19**, 107, 194(1936).

(*6*) CLARK, "Determination of Hydrogen Ions," 3rd ed. (1928), p. 200.

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(*8*) Ibid. **22**, 102, 563(1939).

(*9*) Ind. Eng. Chem., Anal. Ed. **9**, 141(1937).

(*10*) KOLTOFF & STENGER, "Volumetric Analysis," II, 80, (1947); J. Assoc. Offic. Agr. Chemists **22**, 563(1939).

(*11*) KOLTOFF & STENGER, "Volumetric Analysis," II, 78(1947).

(*12*) J. Assoc. Offic. Agr. Chemists **30**, 502 (1947); **31**, 119, 572(1948).

(*13*) Ibid. **32**, 587(1949); **33**, 225(1950).

(*14*) Ibid. **23**, 543(1940); **24**, 100(1941); **31**, 568(1948).

(*15*) Ibid. **24**, 100, 631(1941).

(*16*) Ibid. **19**, 107, 194(1936).

(*17*) Ibid. **25**, 652(1942); J. Research Natl. Bur. Standards **15**, 493(1935), Research Paper No. 843.

(*18*) J. Assoc. Offic. Agr. Chemists **25**, 659 (1942); **27**, 557(1944); **28**, 594(1945); **38**, 382 (1955).

(*19*) Ibid. **22**, 102, 563(1939).

(*20*) J. Chem. Soc. Trans. **57**, 64(1890); J. Soc. Chem. Ind. (1899), 1091; J. Assoc. Offic. Agr. Chemists **24**, 636(1941).

(*21*) J. Assoc. Offic. Agr. Chemists **31**, 573 (1948); **32**, 589(1949).

(*22*) Ibid. **5**, 207(1921).

43. Reference Tables

International atomic weights[a]
1958

43.001

	Symbol	Atomic number	Atomic weight		Symbol	Atomic number	Atomic weight
Actinium	Ac	89	227.	Neodymium	Nd	60	144.27
Aluminum	Al	13	26.98	Neon	Ne	10	20.183
Antimony	Sb	51	121.76	Nickel	Ni	28	58.71
Argon	A	18	39.944	Niobium	Nb	41	92.91
Arsenic	As	33	74.91	Nitrogen	N	7	14.008
Barium	Ba	56	137.36	Osmium	Os	76	190.2
Beryllium	Be	4	9.013	Oxygen	O	8	**16.**
Bismuth	Bi	83	209.00	Palladium	Pd	46	106.4
Boron	B	5	10.82	Phosphorus	P	15	30.975
Bromine	Br	35	79.916	Platinum	Pt	78	195.09
Cadmium	Cd	48	112.41	Polonium	Po	84	210.
Calcium	Ca	20	40.08	Potassium	K	19	39.100
Carbon	C	6	12.011	Praseodymium	Pr	59	140.92
Cerium	Ce	58	140.13	Protactinium	Pa	91	231.
Cesium	Cs	55	132.91	Radium	Ra	88	226.05
Chlorine	Cl	17	35.457	Radon	Rn	86	222.
Chromium	Cr	24	52.01	Rhenium	Re	75	186.22
Cobalt	Co	27	58.94	Rhodium	Rh	45	102.91
Copper	Cu	29	63.54	Rubidium	Rb	37	85.48
Dysprosium	Dy	66	162.51	Ruthenium	Ru	44	101.1
Erbium	Er	68	167.27	Samarium	Sm	62	150.35
Europium	Eu	63	152.0	Scandium	Sc	21	44.96
Fluorine	F	9	19.00	Selenium	Se	34	78.96
Gadolinium	Gd	64	157.26	Silicon	Si	14	28.09
Gallium	Ga	31	69.72	Silver	Ag	47	107.880
Germanium	Ge	32	72.60	Sodium	Na	11	22.991
Gold	Au	79	197.0	Strontium	Sr	38	87.63
Hafnium	Hf	72	178.50	Sulfur	S	16	32.066
Helium	He	2	4.003	Tantalum	Ta	73	180.95
Holmium	Ho	67	164.94	Tellurium	Te	52	127.61
Hydrogen	H	1	1.0080	Terbium	Tb	65	158.93
Indium	In	49	114.82	Thallium	Tl	81	204.39
Iodine	I	53	126.91	Thorium	Th	90	232.05
Iridium	Ir	77	192.2	Thulium	Tm	69	168.94
Iron	Fe	26	55.85	Tin	Sn	50	118.70
Krypton	Kr	36	83.80	Titanium	Ti	22	47.90
Lanthanum	La	57	138.92	Tungsten	W	74	183.86
Lead	Pb	82	207.21	Uranium	U	92	238.07
Lithium	Li	3	6.940	Vanadium	V	23	50.95
Lutecium	Lu	71	174.99	Xenon	Xe	54	131.30
Magnesium	Mg	12	24.32	Ytterbium	Yb	70	173.04
Manganese	Mn	25	54.94	Yttrium	Y	39	88.92
Mercury	Hg	80	200.61	Zinc	Zn	30	65.38
Molybdenum	Mo	42	95.95	Zirconium	Zr	40	91.22

[a] Taken from *J. Am. Chem. Soc.* **80,** 4121 (1958).

43.002 *Various strength solns of the common acids, alkalies, and alcohol*[a]

(a) *Hydrochloric acid solns:* Specification requires not <35% HCl by wt. Sp. gr. = 1.1778 at 15°. Mix with H_2O and dil. to 1 L.

HCL STRENGTH DESIRED	HYDROCHLORIC ACID REQUIRED		
GRAMS PER LITER	GRAMS	ML	
5	14.29	12.13	
10	28.57	24.26	
15	42.85	36.39	
20	57.14	48.52	
36.46	104.17	88.45	1*N* soln
50	142.86	121.29	
100	285.71	242.58	
150	428.57	363.88	
200	571.43	485.17	
222.6	636.00	539.99	Constant boiling
278.4	795.43	675.35	Sp. gr. 1.125
300	857.14	727.75	

(b) *Sulfuric acid solns:* Specification requires not <94% H_2SO_4 by wt. Sp. gr. = 1.835 at 15°. Pour acid into excess of H_2O and dil. to 1 L.

H_2SO_4 STRENGTH DESIRED	SULFURIC ACID REQUIRED		
GRAMS PER LITER	GRAMS	ML	
5	5.32	3.0	
12.5	13.29	7.2	For crude fiber
20	21.28	11.6	
30	31.91	17.4	
40	42.55	23.2	
49	52.13	28.4	1*N* soln
100	106.38	58.0	
150	159.57	87.0	
250	265.96	144.9	
300	319.15	173.9	
400	425.53	231.9	

(c) *Nitric acid solns:* Specification requires not <68% HNO_3 by wt. Sp. gr. = 1.4146 at 15°. 1 ml concd HNO_3 contains ca 0.96 g HNO_3. Mix with H_2O and dil. to 1 L.

HNO_3 STRENGTH DESIRED	NITRIC ACID REQUIRED	
GRAMS PER LITER	GRAMS	ML
5	7.35	5.2
10	14.71	10.4
20	29.41	20.8
30	44.12	31.2
40	58.82	41.6
50	73.53	52.0
63	92.65	65.5
70	102.94	72.8
100	147.06	104.0
150	220.59	156.0
200	294.12	207.9
300	441.18	312.9

[a] Prepared by G. C. Spencer and H. J. Fisher.

Various strength solns of common acids, alkalies, and alcohol—Concluded. **43.002**

(d) *Ammonia solns:* Specification requires not <27% NH_3 by wt. Sp. gr. = 0.9. Mix and dil. to 1 L.

NH_3 STRENGTH DESIRED	REAGENT AMMONIA REQUIRED	
GRAMS PER LITER	GRAMS	ML
5	18.52	20.6
10	37.04	41.1
15	55.55	61.7
20	74.07	82.3
25	92.59	102.9
50	185.18	205.8
75	277.77	308.6
100	370.37	411.5
150	555.55	617.3
200	740.74	823.0

(e) *Sodium hydroxide solns:* Specification requires 95% NaOH in sticks of caustic soda. Dissolve and dil. to 1 L.

NaOH STRENGTH DESIRED	SODIUM HYDROXIDE REQUIRED	
GRAMS PER LITER	GRAMS	
12.5	13.16	For crude fiber
30	31.58	
40	42.11	1*N* soln
50	52.63	
75	78.95	
100	105.26	
150	157.89	
200	210.53	
250	263.16	
300	315.79	

(f) *Alcoholic solns:*[b] Specification requires 95% C_2H_5OH by vol. Sp. gr. = 0.810 at 25°. Mix and dil. to 1 L.

ALCOHOL STRENGTH DESIRED	ALCOHOL REQUIRED	
ML PER LITER	GRAMS	ML
50	42.63	52.6
100	85.26	105.3
150	127.89	157.9
200	170.52	210.5
250	213.16	263.2
300	255.78	315.9
400	341.04	421.1
500	426.32 (proof)	526.3
700	596.84	736.8

[b] Alcohol of any desired strength may be obtained by taking number of ml 95% alcohol equiv. to desired strength and dilg soln to 95 ml. For example: To obtain soln of 70% alcohol, take 70 ml 95% alcohol and dil. to 95 ml.

43.003 *Degrees Brix, specific gravity, and degrees Baumé of sugar solns*[a] *(Plato Table*[b]*)*

Degrees Brix or per cent by weight of sucrose	Specific gravity at 20/20°C.	Specific gravity at 20/4°C.	Degrees Baumé (modulus 145)	Degrees Brix or per cent by weight of sucrose	Specific gravity at 20/20°C.	Specific gravity at 20/4°C.	Degrees Baumé (modulus 145)
0.0	1.00000	0.998234	0.00	9.0	1.03586	1.034029	5.02
0.2	1.00078	0.999010	0.11	9.2	1.03668	1.034850	5.13
0.4	1.00155	0.999786	0.22	9.4	1.03750	1.035671	5.24
0.6	1.00233	1.000563	0.34	9.6	1.03833	1.036494	5.35
0.8	1.00311	1.001342	0.45	9.8	1.03915	1.037318	5.46
1.0	1.00389	1.002120	0.56	10.0	1.03998	1.038143	5.57
1.2	1.00467	1.002897	0.67	10.2	1.04081	1.038970	5.68
1.4	1.00545	1.003675	0.79	10.4	1.04164	1.039797	5.80
1.6	1.00623	1.004453	0.90	10.6	1.04247	1.040626	5.91
1.8	1.00701	1.005234	1.01	10.8	1.04330	1.041456	6.02
2.0	1.00779	1.006015	1.12	11.0	1.04413	1.042288	6.13
2.2	1.00858	1.006796	1.23	11.2	1.04497	1.043121	6.24
2.4	1.00936	1.007580	1.34	11.4	1.04580	1.043954	6.35
2.6	1.01015	1.008363	1.46	11.6	1.04664	1.044788	6.46
2.8	1.01093	1.009148	1.57	11.8	1.04747	1.045625	6.57
3.0	1.01172	1.009934	1.68	12.0	1.04831	1.046462	6.68
3.2	1.01251	1.010721	1.79	12.2	1.04915	1.047300	6.79
3.4	1.01330	1.011510	1.90	12.4	1.04999	1.048140	6.90
3.6	1.01409	1.012298	2.02	12.6	1.05084	1.048980	7.02
3.8	1.01488	1.013089	2.13	12.8	1.05168	1.049822	7.13
4.0	1.01567	1.013881	2.24	13.0	1.05252	1.050665	7.24
4.2	1.01647	1.014673	2.35	13.2	1.05337	1.051510	7.35
4.4	1.01726	1.015467	2.46	13.4	1.05422	1.052356	7.46
4.6	1.01806	1.016261	2.57	13.6	1.05506	1.053202	7.57
4.8	1.01886	1.017058	2.68	13.8	1.05591	1.054050	7.68
5.0	1.01965	1.017854	2.79	14.0	1.05677	1.054900	7.79
5.2	1.02045	1.018652	2.91	14.2	1.05762	1.055751	7.90
5.4	1.02125	1.019451	3.02	14.4	1.05847	1.056602	8.01
5.6	1.02206	1.020251	3.13	14.6	1.05933	1.057455	8.12
5.8	1.02286	1.021053	3.24	14.8	1.06018	1.058310	8.23
6.0	1.02366	1.021855	3.35	15.0	1.06104	1.059165	8.34
6.2	1.02447	1.022659	3.46	15.2	1.06190	1.060022	8.45
6.4	1.02527	1.023463	3.57	15.4	1.06276	1.060880	8.56
6.6	1.02608	1.024270	3.69	15.6	1.06362	1.061738	8.67
6.8	1.02689	1.025077	3.80	15.8	1.06448	1.062598	8.78
7.0	1.02770	1.025885	3.91	16.0	1.06534	1.063460	8.89
7.2	1.02851	1.026694	4.02	16.2	1.06621	1.064324	9.00
7.4	1.02932	1.027504	4.13	16.4	1.06707	1.065188	9.11
7.6	1.03013	1.028316	4.24	16.6	1.06794	1.066054	9.22
7.8	1.03095	1.029128	4.35	16.8	1.06881	1.066921	9.33
8.0	1.03176	1.029942	4.46	17.0	1.06968	1.067789	9.45
8.2	1.03258	1.030757	4.58	17.2	1.07055	1.068658	9.56
8.4	1.03340	1.031573	4.69	17.4	1.07142	1.069529	9.67
8.6	1.03422	1.032391	4.80	17.6	1.07229	1.070400	9.78
8.8	1.03504	1.033209	4.91	17.8	1.07317	1.071273	9.89

[a] Bur. Standards Circ. C440, 1942, pp. 614, 626.
[b] Based upon figures prepared by Kaiserliche Normal-Eichungs-Kommission and accepted by International Commission for Uniform Methods of Sugar Analysis.

Degrees Brix, specific gravity, and degrees Baumé of sugar solns—Continued. **43.003**

DEGREES BRIX OR PER CENT BY WEIGHT OF SUCROSE	SPECIFIC GRAVITY AT 20/20°C.	SPECIFIC GRAVITY AT 20/4°C.	DEGREES BAUMÉ (MODULUS 145)	DEGREES BRIX OR PER CENT BY WEIGHT OF SUCROSE	SPECIFIC GRAVITY AT 20/20°C.	SPECIFIC GRAVITY AT 20/4°C.	DEGREES BAUMÉ (MODULUS 145)
18.0	1.07404	1.072147	10.00	27.0	1.11480	1.112828	14.93
18.2	1.07492	1.073023	10.11	27.2	1.11573	1.113763	15.04
18.4	1.07580	1.073900	10.22	27.4	1.11667	1.114697	15.15
18.6	1.07668	1.074777	10.33	27.6	1.11761	1.115635	15.26
18.8	1.07756	1.075657	10.44	27.8	1.11855	1.116572	15.37
19.0	1.07844	1.076537	10.55	28.0	1.11949	1.117512	15.48
19.2	1.07932	1.077419	10.66	28.2	1.12043	1.118453	15.59
19.4	1.08021	1.078302	10.77	28.4	1.12138	1.119395	15.69
19.6	1.08110	1.079187	10.88	28.6	1.12232	1.120339	15.80
19.8	1.08198	1.080072	10.99	28.8	1.12327	1.121284	15.91
20.0	1.08287	1.080959	11.10	29.0	1.12422	1.122231	16.02
20.2	1.08376	1.081848	11.21	29.2	1.12517	1.123179	16.13
20.4	1.08465	1.082737	11.32	29.4	1.12612	1.124128	16.24
20.6	1.08554	1.083628	11.43	29.6	1.12707	1.125079	16.35
20.8	1.08644	1.084520	11.54	29.8	1.12802	1.126030	16.46
21.0	1.08733	1.085414	11.65	30.0	1.12898	1.126984	16.57
21.2	1.08823	1.086309	11.76	30.2	1.12993	1.127939	16.67
21.4	1.08913	1.087205	11.87	30.4	1.13089	1.128896	16.78
21.6	1.09003	1.088101	11.98	30.6	1.13185	1.129853	16.89
21.8	1.09093	1.089000	12.09	30.8	1.13281	1.130812	17.00
22.0	1.09183	1.089900	12.20	31.0	1.13378	1.131773	17.11
22.2	1.09273	1.090802	12.31	31.2	1.13474	1.132735	17.22
22.4	1.09364	1.091704	12.42	31.4	1.13570	1.133698	17.33
22.6	1.09454	1.092607	12.52	31.6	1.13667	1.134663	17.43
22.8	1.09545	1.093513	12.63	31.8	1.13764	1.135628	17.54
23.0	1.09636	1.094420	12.74	32.0	1.13861	1.136596	17.65
23.2	1.09727	1.095328	12.85	32.2	1.13958	1.137565	17.76
23.4	1.09818	1.096236	12.96	32.4	1.14055	1.138534	17.87
23.6	1.09909	1.097147	13.07	32.6	1.14152	1.139506	17.98
23.8	1.10000	1.098058	13.18	32.8	1.14250	1.140479	18.08
24.0	1.10092	1.098971	13.29	33.0	1.14347	1.141453	18.19
24.2	1.10183	1.099886	13.40	33.2	1.14445	1.142429	18.30
24.4	1.10275	1.100802	13.51	33.4	1.14543	1.143405	18.41
24.6	1.10367	1.101718	13.62	33.6	1.14641	1.144384	18.52
24.8	1.10459	1.102637	13.73	33.8	1.14739	1.145363	18.63
25.0	1.10551	1.103557	13.84	34.0	1.14837	1.146345	18.73
25.2	1.10643	1.104478	13.95	34.2	1.14936	1.147328	18.84
25.4	1.10736	1.105400	14.06	34.4	1.15034	1.148313	18.95
25.6	1.10828	1.106324	14.17	34.6	1.15133	1.149298	19.06
25.8	1.10921	1.107248	14.28	34.8	1.15232	1.150286	19.17
26.0	1.11014	1.108175	14.39	35.0	1.15331	1.151275	19.28
26.2	1.11106	1.109103	14.49	35.2	1.15430	1.152265	19.38
26.4	1.11200	1.110033	14.60	35.4	1.15530	1.153256	19.49
26.6	1.11293	1.110963	14.71	35.6	1.15629	1.154249	19.60
26.8	1.11386	1.111895	14.82	35.8	1.15729	1.155242	19.71

43.003 *Degrees Brix, specific gravity, and degrees Baumé of sugar solns*—Continued.

DEGREES BRIX OR PER CENT BY WEIGHT OF SUCROSE	SPECIFIC GRAVITY AT 20/20°C.	SPECIFIC GRAVITY AT 20/4°C.	DEGREES BAUMÉ (MODULUS 145)	DEGREES BRIX OR PER CENT BY WEIGHT OF SUCROSE	SPECIFIC GRAVITY AT 20/20°C.	SPECIFIC GRAVITY AT 20/4°C.	DEGREES BAUMÉ (MODULUS 145)
36.0	1.15828	1.156238	19.81	45.0	1.20467	1.202540	24.63
36.2	1.15928	1.157235	19.92	45.2	1.20573	1.203603	24.74
36.4	1.16028	1.158233	20.03	45.4	1.20680	1.204668	24.85
36.6	1.16128	1.159233	20.14	45.6	1.20787	1.205733	24.95
36.8	1.16228	1.160233	20.25	45.8	1.20894	1.206801	25.06
37.0	1.16329	1.161236	20.35	46.0	1.21001	1.207870	25.17
37.2	1.16430	1.162240	20.46	46.2	1.21108	1.208940	25.27
37.4	1.16530	1.163245	20.57	46.4	1.21215	1.210013	25.38
37.6	1.16631	1.164252	20.68	46.6	1.21323	1.211086	25.48
37.8	1.16732	1.165259	20.78	46.8	1.21431	1.212162	25.59
38.0	1.16833	1.166269	20.89	47.0	1.21538	1.213238	25.70
38.2	1.16934	1.167281	21.00	47.2	1.21646	1.214317	25.80
38.4	1.17036	1.168293	21.11	47.4	1.21755	1.215395	25.91
38.6	1.17138	1.169307	21.21	47.6	1.21863	1.216476	26.01
38.8	1.17239	1.170322	21.32	47.8	1.21971	1.217559	26.12
39.0	1.17341	1.171340	21.43	48.0	1.22080	1.218643	26.23
39.2	1.17443	1.172359	21.54	48.2	1.22189	1.219729	26.33
39.4	1.17545	1.173379	21.64	48.4	1.22298	1.220815	26.44
39.6	1.17648	1.174400	21.75	48.6	1.22406	1.221904	26.54
39.8	1.17750	1.175423	21.86	48.8	1.22516	1.222995	26.65
40.0	1.17853	1.176447	21.97	49.0	1.22625	1.224086	26.75
40.2	1.17956	1.177473	22.07	49.2	1.22735	1.225180	26.86
40.4	1.18058	1.178501	22.18	49.4	1.22844	1.226274	26.96
40.6	1.18162	1.179527	22.29	49.6	1.22954	1.227371	27.07
40.8	1.18265	1.180560	22.39	49.8	1.23064	1.228469	27.18
41.0	1.18368	1.181592	22.50	50.0	1.23174	1.229567	27.28
41.2	1.18472	1.182625	22.61	50.2	1.23284	1.230668	27.39
41.4	1.18575	1.183660	22.72	50.4	1.23395	1.231770	27.49
41.6	1.18679	1.184696	22.82	50.6	1.23506	1.232874	27.60
41.8	1.18783	1.185734	22.93	50.8	1.23616	1.233979	27.70
42.0	1.18887	1.186773	23.04	51.0	1.23727	1.235085	27.81
42.2	1.18992	1.187814	23.14	51.2	1.23838	1.236194	27.91
42.4	1.19096	1.188856	23.25	51.4	1.23949	1.237303	28.02
42.6	1.19201	1.189901	23.36	51.6	1.24060	1.238414	28.12
42.8	1.19305	1.190946	23.46	51.8	1.24172	1.239527	28.23
43.0	1.19410	1.191993	23.57	52.0	1.24284	1.240641	28.33
43.2	1.19515	1.193041	23.68	52.2	1.24395	1.241757	28.44
43.4	1.19620	1.194090	23.78	52.4	1.24507	1.242873	28.54
43.6	1.19726	1.195141	23.89	52.6	1.24619	1.243992	28.65
43.8	1.19831	1.196193	24.00	52.8	1.24731	1.245113	28.75
44.0	1.19936	1.197247	24.10	53.0	1.24844	1.246234	28.86
44.2	1.20042	1.198303	24.21	53.2	1.24956	1.247358	28.96
44.4	1.20148	1.199360	24.32	53.4	1.25069	1.248482	29.06
44.6	1.20254	1.200420	24.42	53.6	1.25182	1.249609	29.17
44.8	1.20360	1.201480	24.53	53.8	1.25295	1.250737	29.27

Degrees Brix, specific gravity, and degrees Baumé of sugar solns—Continued. 43.003

Degrees Brix or per cent by weight of sucrose	Specific gravity at 20/20°C.	Specific gravity at 20/4°C.	Degrees Baumé (modulus 145)	Degrees Brix or per cent by weight of sucrose	Specific gravity at 20/20°C.	Specific gravity at 20/4°C.	Degrees Baumé (modulus 145)
54.0	1.25408	1.251866	29.38	63.0	1.30657	1.304267	34.02
54.2	1.25521	1.252997	29.48	63.2	1.30778	1.305467	34.12
54.4	1.25635	1.254129	29.59	63.4	1.30898	1.306669	34.23
54.6	1.25748	1.255264	29.69	63.6	1.31019	1.307872	34.33
54.8	1.25862	1.256400	29.80	63.8	1.31139	1.309077	34.43
55.0	1.25976	1.257535	29.90	64.0	1.31260	1.310282	34.53
55.2	1.26090	1.258674	30.00	64.2	1.31381	1.311489	34.63
55.4	1.26204	1.259815	30.11	64.4	1.31502	1.312699	34.74
55.6	1.26319	1.260955	30.21	64.6	1.31623	1.313909	34.84
55.8	1.26433	1.262099	30.32	64.8	1.31745	1.315121	34.94
56.0	1.26548	1.263243	30.42	65.0	1.31866	1.316334	35.04
56.2	1.26663	1.264390	30.52	65.2	1.31988	1.317549	35.14
56.4	1.26778	1.265537	30.63	65.4	1.32110	1.318766	35.24
56.6	1.26893	1.266686	30.73	65.6	1.32232	1.319983	35.34
56.8	1.27008	1.267837	30.83	65.8	1.32354	1.321203	35.45
57.0	1.27123	1.268989	30.94	66.0	1.32476	1.322425	35.55
57.2	1.27239	1.270143	31.04	66.2	1.32599	1.323648	35.65
57.4	1.27355	1.271299	31.15	66.4	1.32722	1.324872	35.75
57.6	1.27471	1.272455	31.25	66.6	1.32844	1.326097	35.85
57.8	1.27587	1.273614	31.35	66.8	1.32967	1.327325	35.95
58.0	1.27703	1.274774	31.46	67.0	1.33090	1.328554	36.05
58.2	1.27819	1.275936	31.56	67.2	1.33214	1.329785	36.15
58.4	1.27936	1.277098	31.66	67.4	1.33337	1.331017	36.25
58.6	1.28052	1.278262	31.76	67.6	1.33460	1.332250	36.35
58.8	1.28169	1.279428	31.87	67.8	1.33584	1.333485	36.45
59.0	1.28286	1.280595	31.97	68.0	1.33708	1.334722	36.55
59.2	1.28404	1.281764	32.07	68.2	1.33832	1.335961	36.66
59.4	1.28520	1.282935	32.18	68.4	1.33957	1.337200	36.76
59.6	1.28638	1.284107	32.28	68.6	1.34081	1.338441	36.86
59.8	1.28755	1.285281	32.38	68.8	1.34205	1.339684	36.96
60.0	1.28873	1.286456	32.49	69.0	1.34330	1.340928	37.06
60.2	1.28991	1.287633	32.59	69.2	1.34455	1.342174	37.16
60.4	1.29109	1.288811	32.69	69.4	1.34580	1.343421	37.26
60.6	1.29227	1.289991	32.79	69.6	1.34705	1.344671	37.36
60.8	1.29346	1.291172	32.90	69.8	1.34830	1.345922	37.46
61.0	1.29464	1.292354	33.00	70.0	1.34956	1.347174	37.56
61.2	1.29583	1.293539	33.10	70.2	1.35081	1.348427	37.66
61.4	1.29701	1.294725	33.20	70.4	1.35207	1.349682	37.76
61.6	1.29820	1.295911	33.31	70.6	1.35333	1.350939	37.86
61.8	1.29940	1.297100	33.41	70.8	1.35459	1.352197	37.96
62.0	1.30059	1.298291	33.51	71.0	1.35585	1.353456	38.06
62.2	1.30178	1.299483	33.61	71.2	1.35711	1.354717	38.16
62.4	1.30298	1.300677	33.72	71.4	1.35838	1.355980	38.26
62.6	1.30418	1.301871	33.82	71.6	1.35964	1.357245	38.35
62.8	1.30537	1.303068	33.92	71.8	1.36091	1.358511	38.45

43.003 *Degrees Brix, specific gravity, and degrees Baumé of sugar solns*—Continued.

Degrees Brix or per cent by weight of sucrose	Specific gravity at 20/20°C.	Specific gravity at 20/4°C.	Degrees Baumé (modulus 145)	Degrees Brix or per cent by weight of sucrose	Specific gravity at 20/20°C.	Specific gravity at 20/4°C.	Degrees Baumé (modulus 145)
72.0	1.36218	1.359778	38.55	81.0	1.42088	1.418374	42.95
72.2	1.36346	1.361047	38.65	81.2	1.42222	1.419711	43.05
72.4	1.36473	1.362317	38.75	81.4	1.42356	1.421049	43.14
72.6	1.36600	1.363590	38.85	81.6	1.42490	1.422390	43.24
72.8	1.36728	1.364864	38.95	81.8	1.42625	1.423730	43.33
73.0	1.36856	1.366139	39.05	82.0	1.42759	1.425072	43.43
73.2	1.36983	1.367415	39.15	82.2	1.42894	1.426416	43.53
73.4	1.37111	1.368693	39.25	82.4	1.43029	1.427761	43.62
73.6	1.37240	1.369973	39.35	82.6	1.43164	1.429109	43.72
73.8	1.37368	1.371254	39.44	82.8	1.43298	1.430457	43.81
74.0	1.37496	1.372536	39.54	83.0	1.43434	1.431807	43.91
74.2	1.37625	1.373820	39.64	83.2	1.43569	1.433158	44.00
74.4	1.37754	1.375105	39.74	83.4	1.43705	1.434511	44.10
74.6	1.37883	1.376392	39.84	83.6	1.43841	1.435866	44.19
74.8	1.38012	1.377680	39.94	83.8	1.43976	1.437222	44.29
75.0	1.38141	1.378971	40.03	84.0	1.44112	1.438579	44.38
75.2	1.38270	1.380262	40.13	84.2	1.44249	1.439938	44.48
75.4	1.38400	1.381555	40.23	84.4	1.44385	1.441299	44.57
75.6	1.38530	1.382851	40.33	84.6	1.44521	1.442661	44.67
75.8	1.38660	1.384148	40.43	84.8	1.44658	1.444024	44.76
76.0	1.38790	1.385446	40.53	85.0	1.44794	1.445388	44.86
76.2	1.38920	1.386745	40.62	85.2	1.44931	1.446754	44.95
76.4	1.39050	1.388045	40.72	85.4	1.45068	1.448121	45.05
76.6	1.39180	1.389347	40.82	85.6	1.45205	1.449491	45.14
76.8	1.39311	1.390651	40.92.	85.8	1.45343	1.450860	45.24
77.0	1.39442	1.391956	41.01	86.0	1.45480	1.452232	45.33
77.2	1.39573	1.393263	41.11	86.2	1.45618	1.453605	45.42
77.4	1.39704	1.394571	41.21	86.4	1.45755	1.454980	45.52
77.6	1.39835	1.395881	41.31	86.6	1.45893	1.456357	45.61
77.8	1.39966	1.397192	41.40	86.8	1.46031	1.457735	45.71
78.0	1.40098	1.398505	41.50	87.0	1.46170	1.459114	45.80
78.2	1.40230	1.399819	41.60	87.2	1.46308	1.460495	45.89
78.4	1.40361	1.401134	41.70	87.4	1.46446	1.461877	45.99
78.6	1.40493	1.402452	41.79	87.6	1.46585	1.463260	46.08
78.8	1.40625	1.403771	41.89	87.8	1.46724	1.464645	46.17
79.0	1.40758	1.405091	41.99	88.0	1.46862	1.466032	46.27
79.2	1.40890	1.406412	42.08	88.2	1.47002	1.467420	46.36
79.4	1.41023	1.407735	42.18	88.4	1.47141	1.468810	46.45
79.6	1.41155	1.409061	42.28	88.6	1.47280	1.470200	46.55
79.8	1.41288	1.410387	42.37	88.8	1.47420	1.471592	46.64
80.0	1.41421	1.411715	42.47	89.0	1.47559	1.472986	46.73
80.2	1.41554	1.413044	42.57	89.2	1.47699	1.474381	46.83
80.4	1.41688	1.414374	42.66	89.4	1.47839	1.475779	46.92
80.6	1.41821	1.415706	42.76	89.6	1.47979	1.477176	47.01
80.8	1.41955	1.417039	42.85	89.8	1.48119	1.478575	47.11

Degrees Brix, specific gravity, and degrees Baumé of sugar solns.—Concluded. **43.003**

DEGREES BRIX OR PER CENT BY WEIGHT OF SUCROSE	SPECIFIC GRAVITY AT 20/20°C.	SPECIFIC GRAVITY AT 20/4°C.	DEGREES BAUMÉ (MODULUS 145)	DEGREES BRIX OR PER CENT BY WEIGHT OF SUCROSE	SPECIFIC GRAVITY AT 20/20°C.	SPECIFIC GRAVITY AT 20/4°C.	DEGREES BAUMÉ (MODULUS 145)
90.0	1.48259	1.479976	47.20	95.0	1.51814	1.515455	49.49
90.2	1.48400	1.481378	47.29	95.2	1.51958	1.516893	49.58
90.4	1.48540	1.482782	47.38	95.4	1.52102	1.518332	49.67
90.6	1.48681	1.484187	47.48	95.6	1.52246	1.519771	49.76
90.8	1.48822	1 485593	47.57	95.8	1.52390	1.521212	49.85
91.0	1.48963	1.487002	47.66	96.0	1.52535	1.522656	49.94
91.2	1.49104	1.488411	47.75	96.2	1.52680	1.524100	50.03
91.4	1.49246	1.489823	47.84	96.4	1.52824	1.525546	50.12
91.6	1.49387	1.491234	47.94	96.6	1.52969	1.526993	50.21
91.8	1.49529	1.492647	48.03	96.8	1.53114	1.528441	50.30
92.0	1.49671	1.494063	48.12	97.0	1.53260	1.529891	50.39
92.2	1.49812	1.495479	48.21	97.2	1.53405	1.531342	50.48
92.4	1.49954	1.496897	48.30	97.4	1.53551	1.532794	50.57
92.6	1.50097	1.498316	48.40	97.6	1.53696	1.534248	50.66
92.8	1.50239	1 499736	48 49	97.8	1.53842	1.535704	50.75
93.0	1.50381	1.501158	48.58	98.0	1.53988	1.537161	50.84
93.2	1.50524	1.502582	48.67	98.2	1.54134	1.538618	50.93
93.4	1.50667	1.504006	48.76	98.4	1.54280	1.540076	51.02
93.6	1.50810	1.505432	48.85	98.6	1.54426	1.541536	51.10
93.8	1.50952	1.506859	48 94	98.8	1.54573	1.542998	51.19
94.0	1.51096	1.508289	49.03	99.0	1.54719	1.544462	51.28
94.2	1.51239	1.509720	49.12	99.2	1.54866	1.545926	51.37
94.4	1.51382	1.511151	49.22	99.4	1.55013	1.547392	51.46
94.6	1.51526	1.512585	49.31	99.6	1.55160	1.548861	51.55
94.8	1.51670	1.514019	49.40	99.8	1.55307	1.550329	51.64
				100.0	1 55454	1.551800	51.73

43.004 *Temperature corrections to readings of saccharometers (standard at 20°C)*

(This table is calcd from data on thermal expansion of sugar solns by Plato,[a] and it is assumed that the instrument is of Jena 16^{III} glass. Table should be used with caution and only for approximate results when temp. differs much from standard temp. or from temp. of surrounding air.)

TEMPERATURE IN DEGREES CENTIGRADE	OBSERVED PERCENTAGE OF SUGAR													
	0	5	10	15	20	25	30	35	40	45	50	55	60	70
	Subtract—													
0	0.30	0.49	0.65	0.77	0.89	0.99	1.08	1.16	1.24	1.31	1.37	1.41	1.44	1.49
5	0.36	0.47	0.56	0.65	0.73	0.80	0.86	0.91	0.97	1.01	1.05	1.08	1.10	1.14
10	0.32	0.38	0.43	0.48	0.52	0.57	0.60	0.64	0.67	0.70	0.72	0.74	0.75	0.77
11	0.31	0.35	0.40	0.44	0.48	0.51	0.55	0.58	0.60	0.63	0.65	0.66	0.68	0.70
12	0.29	0.32	0.36	0.40	0.43	0.46	0.50	0.52	0.54	0.56	0.58	0.59	0.60	0.62
13	0.26	0.29	0.32	0.35	0.38	0.41	0.44	0.46	0.48	0.49	0.51	0.52	0.53	0.55
14	0.24	0.26	0.29	0.31	0.34	0.36	0.38	0.40	0.41	0.42	0.44	0.45	0.46	0.47
15	0.20	0.22	0.24	0.26	0.28	0.30	0.32	0.33	0.34	0.36	0.36	0.37	0.38	0.39
16	0.17	0.18	0.20	0.22	0.23	0.25	0.26	0.27	0.28	0.28	0.29	0.30	0.31	0.32
17	0.13	0.14	0.15	0.16	0.18	0.19	0.20	0.20	0.21	0.21	0.22	0.23	0.23	0.24
18	0.09	0.10	0.10	0.11	0.12	0.13	0.13	0.14	0.14	0.14	0.15	0.15	0.15	0.16
19	0.05	0.05	0.05	0.06	0.06	0.06	0.07	0.07	0.07	0.07	0.08	0.08	0.08	0.08
17.5	0.11	0.12	0.12	0.14	0.15	0.16	0.16	0.17	0.17	0.18	0.18	0.19	0.19	0.20
15.56 (60°F)	0.18	0.20	0.22	0.24	0.26	0.28	0.29	0.30	0.30	0.32	0.33	0.33	0.34	0.34
	Add—													
21	0.04	0.05	0.06	0.06	0.06	0.07	0.07	0.07	0.07	0.08	0.08	0.08	0.08	0.09
22	0.10	0.10	0.11	0.12	0.12	0.13	0.14	0.14	0.15	0.15	0.16	0.16	0.16	0.16
23	0.16	0.16	0.17	0.17	0.19	0.20	0.21	0.21	0.22	0.23	0.24	0.24	0.24	0.24
24	0.21	0.22	0.23	0.24	0.26	0.27	0.28	0.29	0.30	0.31	0.32	0.32	0.32	0.32
25	0.27	0.28	0.30	0.31	0.32	0.34	0.35	0.36	0.38	0.38	0.39	0.39	0.40	0.39
26	0.33	0.34	0.36	0.37	0.40	0.40	0.42	0.44	0.46	0.47	0.47	0.48	0.48	0.48
27	0.40	0.41	0.42	0.44	0.46	0.48	0.50	0.52	0.54	0.54	0.55	0.56	0.56	0.56
28	0.46	0.47	0.49	0.51	0.54	0.56	0.58	0.60	0.61	0.62	0.63	0.64	0.64	0.64
29	0.54	0.55	0.56	0.59	0.61	0.63	0.66	0.68	0.70	0.70	0.71	0.72	0.72	0.72
30	0.61	0.62	0.63	0.66	0.68	0.71	0.73	0.76	0.78	0.78	0.79	0.80	0.80	0.81
35	0.99	1.01	1.02	1.06	1.10	1.13	1.16	1.18	1.20	1.21	1.22	1.22	1.23	1.22
40	1.42	1.45	1.47	1.51	1.54	1.57	1.60	1.62	1.64	1.65	1.65	1.65	1.66	1.65
45	1.91	1.94	1.96	2.00	2.03	2.05	2.07	2.09	2.10	2.10	2.10	2.10	2.10	2.08
50	2.46	2.48	2.50	2.53	2.56	2.57	2.58	2.59	2.59	2.58	2.58	2.57	2.56	2.52
55	3.05	3.07	3.09	3.12	3.12	3.12	3.12	3.11	3.10	3.08	3.07	3.05	3.03	2.97
60	3.69	3.72	3.73	3.73	3.72	3.70	3.67	3.65	3.62	3.60	3.57	3.54	3.50	3.43
27.5	0.43	0.44	0.46	0.48	0.50	0.52	0.54	0.56	0.58	0.58	0.59	0.60	0.60	0.60

[a] Wiss. Abh. Kaiserliche Normal-Eichungs-Kommission, Vol. 2, 1900, p. 140.

Domke table of apparent specific gravity of sucrose solns at 20°C[a] **43.005**

Calcd from tables of Kaiserliche Normal-Eichungs-Kommission and accepted by International Commission for Uniform Methods of Sugar Analysis.

DEGREES BRIX OR PER CENT BY WEIGHT OF SUCROSE	.0	.1	.2	.3	.4	.5	.6	.7	.8	.9
0	1.0000	1.0004	1.0008	1.0012	1.0016	1.0019	1.0023	1.0027	1.0031	1.0035
1	1.0039	1.0043	1.0047	1.0051	1.0055	1.0058	1.0062	1.0066	1.0070	1.0074
2	1.0078	1.0082	1.0086	1.0090	1.0094	1.0098	1.0102	1.0106	1.0109	1.0113
3	1.0117	1.0121	1.0125	1.0129	1.0133	1.0137	1.0141	1.0145	1.0149	1.0153
4	1.0157	1.0161	1.0165	1.0169	1.0173	1.0177	1.0181	1.0185	1.0189	1.0193
5	1.0197	1.0201	1.0205	1.0209	1.0213	1.0217	1.0221	1.0225	1.0229	1.0233
6	1.0237	1.0241	1.0245	1.0249	1.0253	1.0257	1.0261	1.0265	1.0269	1.0273
7	1.0277	1.0281	1.0285	1.0289	1.0294	1.0298	1.0302	1.0306	1.0310	1.0314
8	1.0318	1.0322	1.0326	1.0330	1.0334	1.0338	1.0343	1.0347	1.0351	1.0355
9	1.0359	1.0363	1.0367	1.0371	1.0375	1.0380	1.0384	1.0388	1.0392	1.0396
10	1.0400	1.0404	1.0409	1.0413	1.0417	1.0421	1.0425	1.0429	1.0433	1.0438
11	1.0442	1.0446	1.0450	1.0454	1.0459	1.0463	1.0467	1.0471	1.0475	1.0480
12	1.0484	1.0488	1.0492	1.0496	1.0501	1.0505	1.0509	1.0513	1.0517	1.0522
13	1.0526	1.0530	1.0534	1.0539	1.0543	1.0547	1.0551	1.0556	1.0560	1.0564
14	1.0568	1.0573	1.0577	1.0581	1.0585	1.0589	1.0594	1.0598	1.0603	1.0607
15	1.0611	1.0615	1.0620	1.0624	1.0628	1.0633	1.0637	1.0641	1.0646	1.0650
16	1.0654	1.0659	1.0663	1.0667	1.0672	1.0676	1.0680	1.0685	1.0689	1.0693
17	1.0698	1.0702	1.0706	1.0711	1.0715	1.0719	1.0724	1.0728	1.0733	1.0737
18	1.0741	1.0746	1.0750	1.0755	1.0759	1.0763	1.0768	1.0772	1.0777	1.0781
19	1.0785	1.0790	1.0794	1.0799	1.0803	1.0807	1.0812	1.0816	1.0821	1.0825
20	1.0830	1.0834	1.0839	1.0843	1.0848	1.0852	1.0856	1.0861	1.0865	1.0870
21	1.0874	1.0879	1.0883	1.0888	1.0892	1.0897	1.0901	1.0905	1.0910	1.0915
22	1.0919	1.0924	1.0928	1.0933	1.0937	1.0942	1.0946	1.0951	1.0956	1.0960
23	1.0965	1.0969	1.0974	1.0978	1.0983	1.0987	1.0992	1.0997	1.1001	1.1006
24	1.1010	1.1015	1.1020	1.1024	1.1029	1.1033	1.1038	1.1043	1.1047	1.1052
25	1.1056	1.1061	1.1066	1.1070	1.1075	1.1079	1.1084	1.1089	1.1093	1.1098
26	1.1103	1.1107	1.1112	1.1117	1.1121	1.1126	1.1131	1.1135	1.1140	1.1145
27	1.1149	1.1154	1.1159	1.1163	1.1168	1.1173	1.1178	1.1182	1.1187	1.1192
28	1.1196	1.1201	1.1206	1.1210	1.1215	1.1220	1.1225	1.1229	1.1234	1.1239
29	1.1244	1.1248	1.1253	1.1258	1.1263	1.1267	1.1272	1.1277	1.1282	1.1287
30	1.1291	1.1296	1.1301	1.1306	1.1311	1.1315	1.1320	1.1325	1.1330	1.1334
31	1.1339	1.1344	1.1349	1.1354	1.1359	1.1363	1.1368	1.1373	1.1378	1.1383
32	1.1388	1.1393	1.1397	1.1402	1.1407	1.1412	1.1417	1.1422	1.1427	1.1432
33	1.1436	1.1441	1.1446	1.1451	1.1456	1.1461	1.1466	1.1471	1.1476	1.1481
34	1.1486	1.1490	1.1495	1.1500	1.1505	1.1510	1.1515	1.1520	1.1525	1.1530
35	1.1535	1.1540	1.1545	1.1550	1.1555	1.1560	1.1565	1.1570	1.1575	1.1580
36	1.1585	1.1590	1.1595	1.1600	1.1605	1.1610	1.1615	1.1620	1.1625	1.1630
37	1.1635	1.1640	1.1645	1.1650	1.1655	1.1660	1.1665	1.1670	1.1675	1.1680
38	1.1685	1.1690	1.1696	1.1701	1.1706	1.1711	1.1716	1.1721	1.1726	1.1731
39	1.1736	1.1741	1.1746	1.1752	1.1757	1.1762	1.1767	1.1772	1.1777	1.1782
40	1.1787	1.1793	1.1798	1.1803	1.1808	1.1813	1.1818	1.1824	1.1829	1.1834
41	1.1839	1.1844	1.1849	1.1855	1.1860	1.1865	1.1870	1.1875	1.1881	1.1886
42	1.1891	1.1896	1.1901	1.1907	1.1912	1.1917	1.1922	1.1928	1.1933	1.1938
43	1.1943	1.1949	1.1954	1.1959	1.1964	1.1970	1.1975	1.1980	1.1985	1.1991
44	1.1996	1.2001	1.2007	1.2012	1.2017	1.2023	1.2028	1.2033	1.2039	1.2044

[a] *Z. Ver. deut. Zucker-Ind.*, 62, 306 (1912).

43.005 *Domke table of apparent specific gravity of sucrose solns at 20°C*—Concluded.

Degrees Brix or per cent by weight of sucrose	.0	.1	.2	.3	.4	.5	.6	.7	.8	.9
45	1.2049	1.2054	1.2060	1.2065	1.2070	1.2076	1.2081	1.2087	1.2092	1.2097
46	1.2102	1.2108	1.2113	1.2118	1.2124	1.2129	1.2135	1.2140	1.2146	1.2151
47	1.2156	1.2162	1.2167	1.2173	1.2178	1.2184	1.2189	1.2194	1.2200	1.2205
48	1.2211	1.2216	1.2222	1.2227	1.2232	1.2238	1.2243	1.2249	1.2254	1.2260
49	1.2265	1.2271	1.2276	1.2282	1.2287	1.2293	1.2298	1.2304	1.2309	1.2315
50	1.2320	1.2326	1.2331	1.2337	1.2342	1.2348	1.2353	1.2359	1.2364	1.2370
51	1.2376	1.2381	1.2387	1.2392	1.2398	1.2403	1.2409	1.2415	1.2420	1.2426
52	1.2431	1.2437	1.2442	1.2448	1.2454	1.2459	1.2465	1.2471	1.2476	1.2482
53	1.2487	1.2493	1.2499	1.2504	1.2510	1.2516	1.2521	1.2527	1.2533	1.2538
54	1.2544	1.2550	1.2555	1.2561	1.2567	1.2572	1.2578	1.2584	1.2589	1.2595
55	1.2601	1.2606	1.2612	1.2618	1.2624	1.2629	1.2635	1.2641	1.2647	1.2652
56	1.2658	1.2664	1.2670	1.2675	1.2681	1.2687	1.2693	1.2698	1.2704	1.2710
57	1.2716	1.2721	1.2727	1.2733	1.2739	1.2745	1.2750	1.2756	1.2762	1.2768
58	1.2774	1.2779	1.2785	1.2791	1.2797	1.2803	1.2809	1.2815	1.2821	1.2826
59	1.2832	1.2838	1.2844	1.2850	1.2856	1.2861	1.2867	1.2873	1.2879	1.2885
60	1.2891	1.2897	1.2903	1.2909	1.2914	1.2920	1.2926	1.2932	1.2938	1.2944
61	1.2950	1.2956	1.2962	1.2968	1.2974	1.2980	1.2986	1.2992	1.2998	1.3004
62	1.3010	1.3015	1.3021	1.3027	1.3033	1.3039	1.3045	1.3051	1.3057	1.3063
63	1.3069	1.3075	1.3081	1.3087	1.3093	1.3100	1.3106	1.3112	1.3118	1.3124
64	1.3130	1.3136	1.3142	1.3148	1.3154	1.3160	1.3166	1.3172	1.3178	1.3184
65	1.3190	1.3197	1.3203	1.3209	1.3215	1.3221	1.3227	1.3233	1.3239	1.3245
66	1.3252	1.3258	1.3264	1.3270	1.3276	1.3282	1.3288	1.3295	1.3301	1.3307
67	1.3313	1.3319	1.3325	1.3332	1.3338	1.3344	1.3350	1.3356	1.3363	1.3369
68	1.3375	1.3381	1.3387	1.3394	1.3400	1.3406	1.3412	1.3418	1.3425	1.3431
69	1.3437	1.3443	1.3450	1.3456	1.3462	1.3468	1.3475	1.3481	1.3487	1.3494
70	1.3500	1.3506	1.3512	1.3519	1.3525	1.3531	1.3538	1.3544	1.3550	1.3557
71	1.3563	1.3569	1.3575	1.3582	1.3588	1.3594	1.3601	1.3607	1.3614	1.3620
72	1.3626	1.3633	1.3639	1.3645	1.3652	1.3658	1.3664	1.3671	1.3677	1.3684
73	1.3690	1.3696	1.3703	1.3709	1.3716	1.3722	1.3729	1.3735	1.3741	1.3748
74	1.3754	1.3761	1.3767	1.3774	1.3780	1.3786	1.3793	1.3799	1.3806	1.3812
75	1.3819	1.3825	1.3832	1.3838	1.3845	1.3851	1.3858	1.3864	1.3871	1.3877
76	1.3884	1.3890	1.3897	1.3903	1.3910	1.3916	1.3923	1.3929	1.3936	1.3942
77	1.3949	1.3955	1.3962	1.3969	1.3975	1.3982	1.3988	1.3995	1.4001	1.4008
78	1.4015	1.4021	1.4028	1.4034	1.4041	1.4048	1.4054	1.4061	1.4067	1.4074
79	1.4081	1.4087	1.4094	1.4101	1.4107	1.4114	1.4121	1.4127	1.4134	1.4140
80	1.4147	1.4154	1.4160	1.4167	1.4174	1.4180	1.4187	1.4194	1.4201	1.4207
81	1.4214	1.4221	1.4227	1.4234	1.4241	1.4247	1.4254	1.4261	1.4268	1.4274
82	1.4281	1.4288	1.4295	1.4301	1.4308	1.4315	1.4322	1.4328	1.4335	1.4342
83	1.4349	1.4355	1.4362	1.4369	1.4376	1.4383	1.4389	1.4396	1.4403	1.4410
84	1.4417	1.4423	1.4430	1.4437	1.4444	1.4451	1.4458	1.4464	1.4471	1.4478
85	1.4485	1.4492	1.4499	1.4505	1.4512	1.4519	1.4526	1.4533	1.4540	1.4547
86	1.4554	1.4560	1.4567	1.4574	1.4581	1.4588	1.4595	1.4602	1.4609	1.4616
87	1.4623	1.4629	1.4636	1.4643	1.4650	1.4657	1.4664	1.4671	1.4678	1.4685
88	1.4692	1.4699	1.4706	1.4713	1.4720	1.4727	1.4734	1.4741	1.4748	1.4755
89	1.4762	1.4769	1.4776	1.4783	1.4790	1.4797	1.4804	1.4811	1.4818	1.4825
90	1.4832									

Jackson-Mathews table of densities of levulose solns and mean density and expansion coefficients between 20° and 25°C[a] 43.006

(All wts corrected to vac.)

Levulose, per cent	D_4^{20}	D_4^{25}	$-\Delta D/\Delta t$	$\Delta v/\Delta t$
			$\times 10^{-6}$	$\times 10^{-6}$
0	0.99823	0.99708	231	231
1	1.00214	1.00095	238	237
2	1.00607	1.00484	245	243
3	1.01003	1.00877	252	249
4	1.01402	1.01272	259	255
5	1.01803	1.01670	266	261
6	1.02207	1.02071	273	267
7	1.02614	1.02475	280	273
8	1.03024	1.02881	287	278
9	1.03437	1.03290	294	284
10	1.03853	1.03702	301	290
11	1.04271	1.04118	308	295
12	1.04692	1.04535	315	300
13	1.05116	1.04955	323	307
14	1.05543	1.05378	330	313
15	1.05972	1.05804	337	318
16	1.06405	1.06233	345	324
17	1.06840	1.06664	352	329
18	1.07278	1.07098	360	336
19	1.07719	1.07535	367	341
20	1.08162	1.07975	375	347
			$\times 10^{-5}$	$\times 10^{-5}$
21	1.08606	1.0842	38	35
22	1.09055	1.0886	38	35
23	1.09507	1.0931	39	36
24	1.09962	1.0976	40	36
25	1.10420	1.1022	41	37
26	1.1088	1.10675	41	37
27	1.11345	1.11135	42	38
28	1.1181	1.1160	43	38
29	1.1229	1.1207	43	39
30	1.1276	1.1254	44	39
31	1.1324	1.13015	45	40
32	1.1372	1.1349	46	40
33	1.14205	1.1397	46	40
34	1.1469	1.1446	47	41
35	1.15185	1.1495	48	41

Levulose, per cent	D_4^{20}	D_4^{25}	$-\Delta D/\Delta t$	$\Delta v/\Delta t$
			$\times 10^{-5}$	$\times 10^{-5}$
36	1.1568	1.1544	48	42
37	1.1618	1.1593	49	42
38	1.1668	1.1643	50	43
39	1.1718	1.1693	50	43
40	1.1769	1.17435	51	43
41	1.1820	1.1794	52	44
42	1.1872	1.1845	53	44
43	1.1923	1.1897	53	45
44	1.1975	1.19485	54	45
45	1.2028	1.20005	55	45
46	1.20805	1.2053	55	46
47	1.2134	1.2106	56	46
48	1.2187	1.2159	57	46
49	1.2241	1.2212	57	47
50	1.2295	1.2266	58	47
51	1.2349	1.2320	59	47
52	1.2404	1.2374	59	48
53	1.2459	1.2429	60	48
54	1.2514	1.2484	60	48
55	1.2570	1.2539	61	49
56	1.2626	1.2595	62	49
57	1.2682	1.2651	62	49
58	1.2739	1.2707	63	50
59	1.2796	1.2764	64	50
60	1.2853	1.2821	64	50
61	1.2911	1.2878	65	50
62	1.2969	1.2936	66	51
63	1.3027	1.2994	66	51
64	1.3086	1.3052	67	51
65	1.3145	1.3111	67	51
66	1.3204	1.3170	68	51
67	1.3263	1.3229	69	52
68	1.3323	1.3289	69	52
69	1.3384	1.3349	70	52
70	1.3444	1.3409	70	52
71	1.3505	1.3470	71	53

[a] *J. Research, NBS,* **8,** 437 (1932), RP 426; Circ. 440 NBS.

43.007 *Refractive indices of sucrose solns at 20°C*[a]
(International Scale, 1936)[b]

Refractive Index at 20°	Sucrose, per cent	Refractive Index at 20°	Sucrose, per cent	Refractive Index at 20°	Sucrose, per cent	Refractive Index at 20°	Sucrose, per cent	Refractive Index at 20°	Sucrose, per cent
1.33299	0.0	1.34629	9.0	1.36053	18.0	1.3758	27.0	1.3920	36.0
.33328	0.2	.34660	9.2	.36086	18.2	.3761	27.2	.3924	36.2
.33357	0.4	.34691	9.4	.36119	18.4	.3765	27.4	.3928	36.4
.33385	0.6	.34721	9.6	.36152	18.6	.3768	27.6	.3931	36.6
.33414	0.8	.34752	9.8	.36185	18.8	.3772	27.8	.3935	36.8
.33443	1.0	.34783	10.0	.36218	19.0	.3775	28.0	.3939	37.0
.33472	1.2	.34814	10.2	.36251	19.2	.3779	28.2	.3943	37.2
.33501	1.4	.34845	10.4	.36284	19.4	.3782	28.4	.3947	37.4
.33530	1.6	.34875	10.6	.36318	19.6	.3786	28.6	.3950	37.6
.33559	1.8	.34906	10.8	.36351	19.8	.3789	28.8	.3954	37.8
.33588	2.0	.34937	11.0	.36384	20.0	.3793	29.0	.3958	38.0
.33617	2.2	.34968	11.2	.36417	20.2	.3797	29.2	.3962	38.2
.33646	2.4	.34999	11.4	.36451	20.4	.3800	29.4	.3966	38.4
.33675	2.6	.35031	11.6	.36484	20.6	.3804	29.6	.3970	38.6
.33704	2.8	.35062	11.8	.36518	20.8	.3807	29.8	.3974	38.8
.33733	3.0	.35093	12.0	.36551	21.0	.3811	30.0	.3978	39.0
.33762	3.2	.35124	12.2	.36585	21.2	.3815	30.2	.3982	39.2
.33792	3.4	.35156	12.4	.36618	21.4	.3818	30.4	.3986	39.4
.33821	3.6	.35187	12.6	.36652	21.6	.3822	30.6	.3989	39.6
.33851	3.8	.35219	12.8	.36685	21.8	.3825	30.8	.3993	39.8
.33880	4.0	.35250	13.0	.36719	22.0	.3829	31.0	.3997	40.0
.33909	4.2	.35282	13.2	.36753	22.2	.3833	31.2	.4001	40.2
.33939	4.4	.35313	13.4	.36787	22.4	.3836	31.4	.4005	40.4
.33968	4.6	.35345	13.6	.36820	22.6	.3840	31.6	.4008	40.6
.33998	4.8	.35376	13.8	.36854	22.8	.3843	31.8	.4012	40.8
.34027	5.0	.35408	14.0	.36888	23.0	.3847	32.0	.4016	41.0
.34057	5.2	.35440	14.2	.36922	23.2	.3851	32.2	.4020	41.2
.34087	5.4	.35472	14.4	.36956	23.4	.3854	32.4	.4024	41.4
.34116	5.6	.35503	14.6	.36991	23.6	.3858	32.6	.4028	41.6
.34146	5.8	.35535	14.8	.37025	23.8	.3861	32.8	.4032	41.8
.34176	6.0	.35567	15.0	.37059	24.0	.3865	33.0	.4036	42.0
.34206	6.2	.35599	15.2	.3709	24.2	.3869	33.2	.4040	42.2
.34236	6.4	.35631	15.4	.3713	24.4	.3872	33.4	.4044	42.4
.34266	6.6	.35664	15.6	.3716	24.6	.3876	33.6	.4048	42.6
.34296	6.8	.35696	15.8	.3720	24.8	.3879	33.8	.4052	42.8
.34326	7.0	.35728	16.0	.3723	25.0	.3883	34.0	.4056	43.0
.34356	7.2	.35760	16.2	.3726	25.2	.3887	34.2	.4060	43.2
.34386	7.4	.35793	16.4	.3730	25.4	.3891	34.4	.4064	43.4
.34417	7.6	.35825	16.6	.3733	25.6	.3894	34.6	.4068	43.6
.34447	7.8	.35858	16.8	.3737	25.8	.3898	34.8	.4072	43.8
.34477	8.0	.35890	17.0	.3740	26.0	.3902	35.0	.4076	44.0
.34507	8.2	.35923	17.2	.3744	26.2	.3906	35.2	.4080	44.2
.34538	8.4	.35955	17.4	.3747	26.4	.3909	35.4	.4084	44.4
.34568	8.6	.35988	17.6	.3751	26.6	.3913	35.6	.4088	44.6
.34599	8.8	.36020	17.8	.3754	26.8	.3916	35.8	.4092	44.8

[a] The values in this table for the range 0 to 49.8% sucrose are in accordance with the International Scale of Refractive Indices of Sucrose at 20°C, 1936 adopted as official at the 1938 meeting of the Association. Values of indices for range 0–24% sucrose are given to five decimal places, those 24.2 to 49.8% to four decimal places. Values for range 50 to 85% are those adopted as official at the 1959 meeting and are given to five decimal places.

[b] *Intern. Sugar J.* 39, 225 (1937).

Refractive indices of sucrose solns at 20°C—Concluded. **43.007**

REFRACTIVE INDEX AT 20°	SUCROSE, PER CENT	REFRACTIVE INDEX AT 20°	SUCROSE, PER CENT	REFRACTIVE INDEX AT 20°	SUCROSE, PER CENT	REFRACTIVE INDEX AT 20°	SUCROSE, PER CENT	REFRACTIVE INDEX AT 20°	SUCROSE, PER CENT
1.4096	45.0	1.42646	53.0	1.44420	61.0	1.46299	69.0	1.48288	77.0
.4100	45.2	.42689	53.2	.44465	61.2	.46347	69.2	.48339	77.2
.4104	45.4	.42733	53.4	.44511	61.4	.46396	69.4	.48390	77.4
.4109	45.6	.42776	53.6	.44557	61.6	.46444	69.6	.48442	77.6
.4113	45.8	.42819	53.8	.44603	61.8	.46493	69.8	.48493	77.8
.4117	46.0	.42862	54.0	.44649	62.0	.46541	70.0	.48544	78.0
.4121	46.2	.42906	54.2	.44695	62.2	.46590	70.2	.48596	78.2
.4125	46.4	.42949	54.4	.44741	62.4	.46639	70.4	.48648	78.4
.4129	46.6	.42993	54.6	.44787	62.6	.46688	70.6	.48699	78.6
.4133	46.8	.43036	54.8	.44833	62.8	.46737	70.8	.48751	78.8
.4137	47.0	.43080	55.0	.44879	63.0	.46786	71.0	.48803	79.0
.4141	47.2	.43124	55.2	.44926	63.2	.46835	71.2	.48855	79.2
.4145	47.4	.43168	55.4	.44972	63.4	.46884	71.4	.48907	79.4
.4150	47.6	.43211	55.6	.45019	63.6	.46933	71.6	.48959	79.6
.4154	47.8	.43255	55.8	.45065	63.8	.46982	71.8	.49011	79.8
.4158	48.0	.43299	56.0	.45112	64.0	.47032	72.0	.49063	80.0
.4162	48.2	.43343	56.2	.45158	64.2	.47081	72.2	.49115	80.2
.4166	48.4	.43387	56.4	.45205	64.4	.47131	72.4	.49167	80.4
.4171	48.6	.43432	56.6	.45252	64.6	.47180	72.6	.49220	80.6
.4175	48.8	.43476	56.8	.45299	64.8	.47230	72.8	.49272	80.8
.4179	49.0	.43520	57.0	.45346	65.0	.47279	73.0	.49325	81.0
.4183	49.2	.43564	57.2	.45393	65.2	.47329	73.2	.49377	81.2
.4187	49.4	.43609	57.4	.45440	65.4	.47379	73.4	.49430	81.4
.4192	49.6	.43653	57.6	.45487	65.6	.47429	73.6	.49483	81.6
.4196	49.8	.43698	57.8	.45534	65.8	.47479	73.8	.49536	81.8
.42008	50.0	.43742	58.0	.45581	66.0	.47529	74.0	.49589	82.0
.42050	50.2	.43787	58.2	.45629	66.2	.47579	74.2	.49641	82.2
.42092	50.4	.43832	58.4	.45676	66.4	.47629	74.4	.49695	82.4
.42135	50.6	.43877	58.6	.45724	66.6	.47679	74.6	.49748	82.6
.42177	50.8	.43922	58.8	.45771	66.8	.47730	74.8	.49801	82.8
.42219	51.0	.43966	59.0	.45819	67.0	.47780	75.0	.49854	83.0
.42261	51.2	.44011	59.2	.45867	67.2	.47831	75.2	.49907	83.2
.42304	51.4	.44057	59.4	.45914	67.4	.47881	75.4	.49961	83.4
.42347	51.6	.44102	59.6	.45962	67.6	.47932	75.6	.50014	83.6
.42389	51.8	.44147	59.8	.46010	67.8	.47982	75.8	.50068	83.8
.42432	52.0	.44192	60.0	.46058	68.0	.48033	76.0	.50121	84.0
.42475	52.2	.44238	60.2	.46106	68.2	.48084	76.2	.50175	84.2
.42517	52.4	.44283	60.4	.46154	68.4	.48135	76.4	.50229	84.4
.42560	52.6	.44328	60.6	.46202	68.6	.48186	76.6	.50283	86.6
.42603	52.8	.44374	60.8	.46251	68.8	.48237	76.8	.50337	84.8
								.50391	85.0

43.008 *Corrections for detg % sucrose in sugar solns by means of either Abbé or immersion refractometer when readings are made at temps other than 20°C*[a]

(International Temperature Correction Table, 1936)[a]

TEMP. °C	PER CENT SUCROSE 0	5	10	15	20	25	30	40	50	60	70
	Subtract from the per cent sucrose										
10	0.50	0.54	0.58	0.61	0.64	0.66	0.68	0.72	0.74	0.76	0.79
11	.46	.49	.53	.55	.58	.60	.62	.65	.67	.69	.71
12	.42	.45	.48	.50	.52	.54	.56	.58	.60	.61	.63
13	.37	.40	.42	.44	.46	.48	.49	.51	.53	.54	.55
14	.33	.35	.37	.39	.40	.41	.42	.44	.45	.46	.48
15	.27	.29	.31	.33	.34	.34	.35	.37	.38	.39	.40
16	.22	.24	.25	.26	.27	.28	.28	.30	.30	.31	.32
17	.17	.18	.19	.20	.21	.21	.21	.22	.23	.23	.24
18	.12	.13	.13	.14	.14	.14	.14	.15	.15	.16	.16
19	.06	.06	.06	.07	.07	.07	.07	.08	.08	.08	.08
	Add to the per cent sucrose										
21	0.06	0.07	0.07	0.07	0.07	0.08	0.08	0.08	0.08	0.08	0.08
22	.13	.13	.14	.14	.15	.15	.15	.15	.16	.16	.16
23	.19	.20	.21	.22	.22	.23	.23	.23	.24	.24	.24
24	.26	.27	.28	.29	.30	.30	.31	.31	.31	.32	.32
25	.33	.35	.36	.37	.38	.38	.39	.40	.40	.40	.40
26	.40	.42	.43	.44	.45	.46	.47	.48	.48	.48	.48
27	.48	.50	.52	.53	.54	.55	.55	.56	.56	.56	.56
28	.56	.57	.60	.61	.62	.63	.63	.64	.64	.64	.64
29	.64	.66	.68	.69	.71	.72	.72	.73	.73	.73	.73
30	.72	.74	.77	.78	.79	.80	.80	.81	.81	.81	.81

[a] *Intern. Sugar J.*, **39**, 24s (1937).

Table for detg % sucrose in sugar solns from readings of Zeiss immersion refractometer at 20°C[a] 43.009

SCALE READING[b] 20°C.	n_D^{20}	SUCROSE PER CENT	SCALE READING[b] 20°C.	n_D^{20}	SUCROSE PER CENT	SCALE READING[b] 20°C.	n_D^{20}	SUCROSE PER CENT
14.47	1.33299	0	45	1.34463	7.91	76	1.35606	15.24
15	3320	0.15	46	4500	8.15	77	5642	15.47
16	3358	0.41	47	4537	8.39	78	5678	15.69
17	3397	0.68	48	4575	8.64	79	5714	15.91
18	3435	0.94	49	4612	8.89	80	5750	16.14
19	3474	1.21	50	4650	9.13	81	5786	16.36
20	3513	1.48	51	4687	9.38	82	5822	16.58
21	3551	1.74	52	4724	9.62	83	5858	16.81
22	3590	2.01	53	4761	9.86	84	5894	17.03
23	3628	2.27	54	4798	10.10	85	5930	17.25
24	3667	2.54	55	4836	10.34	86	5966	17.47
25	3705	2.80	56	4873	10.58	87	6002	17.69
26	3743	3.07	57	4910	10.82	88	6038	17.91
27	3781	3.33	58	4947	11.06	89	6074	18.12
28	3820	3.59	59	4984	11.30	90	6109	18.34
29	3858	3.85	60	5021	11.54	91	6145	18.56
30	3896	4.11	61	5058	11.78	92	6181	18.78
31	3934	4.36	62	5095	12.01	93	6217	19.00
32	3972	4.62	63	5132	12.25	94	6252	19.21
33	4010	4.88	64	5169	12.48	95	6287	19.42
34	4048	5.14	65	5205	12.72	96	6323	19.63
35	4086	5.40	66	5242	12.95	97	6359	19.85
36	4124	5.65	67	5279	13.18	98	6394	20.06
37	4162	5.91	68	5316	13.41	99	6429	20.27
38	4199	6.16	69	5352	13.64	100	6464	20.48
39	4237	6.41	70	5388	13.87	101	6500	20.69
40	4275	6.66	71	5425	14.10	102	6535	20.90
41	4313	6.91	72	5461	14.33	103	6570	21.11
42	4350	7.16	73	5497	14.56	104	6605	21.32
43	4388	7.41	74	5533	14.79	105	6640	21.53
44	4426	7.66	75	5569	15.01			

[a] Values in this table were calcd by J. A. Mathews from five-place indices of Schönrock as given by Landt, *Z. Ver. deut. Zucker-Ind.*, 83, 692 (1933).

[b] Scale readings refer only to scale of arbitrary units proposed by Pulfrich, *Z. angew. Chem.*, p. 1168 (1899). According to this scale 14.5 = 1.33300, 50.0 = 1.34650, and 100.0 = 1.36464. If immersion refractometer used is calibrated according to another arbitrary scale, readings must be converted into refractive indices before this table is used to det. % sugar.

43.010 *Refractive indices of dextrose, levulose, invert sugar, and raffinose hydrate solns at 20°C*

PER CENT BY WEIGHT IN AIR	REFRACTIVE INDEX			
	DEXTROSE[a]	LEVULOSE	INVERT SUGAR[a]	RAFFINOSE HYDRATE[b]
0	1.33299	1.33299	1.33299	1.33299
1	1.33442	1.33441	1.33441	1.33422
2	1.33586	1.33583	1.33583	1.33546
3	1.33731	1.33727	1.33727	1.33671
4	1.33877	1.33872	1.33872	1.33797
5	1.34024	1.34017	1.34018	1.33924
6	1.34173	1.34164	1.34165	1.34052
7	1.34322	1.34312	1.34313	1.34181
8	1.34472	1.34461	1.34462	1.34311
9	1.34623	1.34611	1.34612	1.34443
10	1.34775	1.34762	1.34764	1.34576
11	1.34928	1.34914	1.34916	1.34709
12	1.35082	1.35067	1.35070	1.34844
13	1.35237	1.35221	1.35225	1.34979
14	1.35393	1.35377	1.35381	1.35116
15	1.35551	1.35534	1.35538	1.35253
16	1.35710	1.35692	1.35696	1.35391
17	1.35870	1.35851	1.35856	1.35530
18	1.36031	1.36011	1.36016	1.35670
19	1.36193	1.36172	1.36178	1.35811
20	1.36356	1.36335	1.36341	1.35953
21	1.36520	1.36499	1.36506	1.36096
22	1.36685	1.36664	1.36671	1.36239
23	1.36852	1.36830	1.36838	1.36384
24	1.37020	1.36998	1.37006	1.36529
25	1.37189	1.37167	1.37175	1.36676
26	1.37359	1.37337	1.37345	1.36824
27	1.37530	1.37508	1.37517	1.36972
28	1.37702	1.37681	1.37690	1.37121
29	1.37876	1.37855	1.37864	1.37272
30	1.38051	1.38030	1.38040	1.37424
31	1.38228	1.38207	1.38217	1.37577
32	1.38406	1.38385	1.38395	1.37730
33	1.38585	1.38564	1.38574	1.37884
34	1.38765	1.38745	1.38755	1.38040
35	1.38946	1.38927	1.38937	1.38197
36	1.39129	1.39110	1.39120	1.38356
37	1.39313	1.39295	1.39305	1.38516
38	1.39498	1.39481	1.39491	1.38677
39	1.39684	1.39668	1.39678	1.38840
40	1.39872	1.39857	1.39866	1.39004
41	1.40061	1.40047	1.40056	
42	1.40251	1.40238	1.40248	
43	1.40443	1.40431	1.40440	
44	1.40636	1.40625	1.40634	
45	1.40831	1.40821	1.40830	
46	1.41028	1.41018	1.41026	
47	1.41226	1.41216	1.41225	

[a] Zerban and Martin, *J. Assoc. Offic. Agr. Chemists*, **27**, 295 (1944). Dextrose values graphically smoothed, Young and Jones, *Ibid.*, 37, 932 (1954).
[b] Zerban and Martin, *Ibid.*, **34**, 808 (1951).

Refractive indices of dextrose, levulose, invert sugar, and raffinose hydrate solns at 20°C—Concluded. 43.010

PER CENT BY WEIGHT IN AIR	REFRACTIVE INDEX			
	DEXTROSE[a]	LEVULOSE	INVERT SUGAR[a]	RAFFINOSE HYDRATE[b]
48	1.41425	1.41415	1.41424	
49	1.41625	1.41616	1.41625	
50	1.41826	1.41819	1.41827	
51	1.42029	1.42022	1.42031	
52	1.42233	1.42228	1.42236	
53	1.42439	1.42434	1.42443	
54	1.42646	1.42642	1.42650	
55	1.42855	1.42851	1.42860	
56	1.43065	1.43062	1.43070	
57	1.43276	1.43273	1.43283	
58	1.43488	1.43487	1.43496	
59	1.43702	1.43701	1.43711	
60	1.43918	1.43917	1.43928	
61	1.44135	1.44135	1.44146	
62	1.44354	1.44353	1.44365	
63	1.44574	1.44573	1.44586	
64	1.44796	1.44794	1.44808	
65	1.45019	1.45017	1.45032	
66	1.45244	1.45241	1.45257	
67	1.45470	1.45466	1.45484	
68	1.45697	1.45693	1.45712	
69	1.45926	1.45921	1.45941	
70	1.46156	1.46150	1.46172	
71	1.46388	1.46380	1.46405	
72	1.46621	1.46612	1.46639	
73	1.46856	1.46845	1.46874	
74	1.47092	1.47079	1.47111	
75	1.47330	1.47315	1.47350	
76	1.47569	1.47551	1.47590	
77	1.47810	1.47789	1.47831	
78	1.48052	1.48028	1.48074	
79	1.48296	1.48269	1.48319	
80	1.48542	1.48510	1.48564	
81		1.48753		
82		1.48997		
83		1.49242		
84		1.49488		
85		1.49735		
86		1.49984		
87		1.50233		
88		1.50484		
89		1.50736		
90		1.50988		

43.011 *Munson and Walker table for calcg dextrose, invert sugar alone, invert sugar in presence of sucrose (0.4 g and 2 g total sugar), lactose, lactose and sucrose (2 mixts), and maltose (crystd)*[a]

(Applicable when Cu_2O is weighed directly)
(Expressed in mg)

CUPROUS OXIDE (Cu_2O)	DEXTROSE (*d*-GLUCOSE)	INVERT SUGAR	INVERT SUGAR AND SUCROSE		LACTOSE	LACTOSE AND SUCROSE		MALTOSE	CUPROUS OXIDE (Cu_2O)
			0.4 gram total sugar	2 grams total sugar	$C_{12}H_{22}O_{11}+H_2O$	1 lactose, 4 sucrose	1 lactose, 12 sucrose	$C_{12}H_{22}O_{11}+H_2O$	
10	4.0	4.5	1.6	....	6.3	6.1	....	6.2	10
12	4.9	5.4	2.5	....	7.5	7.3	....	7.9	12
14	5.7	6.3	3.4	....	8.8	8.5	....	9.5	14
16	6.6	7.2	4.3	....	10.0	9.7	....	11.2	16
18	7.5	8.1	5.2	....	11.3	10.9	....	12.9	18
20	8.3	8.9	6.1	....	12.5	12.1	....	14.6	20
22	9.2	9.8	7.0	....	13.8	13.3	....	16.2	22
24	10.0	10.7	7.9	....	15.0	14.5	....	17.9	24
26	10.9	11.6	8.8	....	16.3	15.8	....	19.6	26
28	11.8	12.5	9.7	....	17.6	17.0	....	21.2	28
30	12.6	13.4	10.7	4.3	18.8	18.2	....	22.9	30
32	13.5	14.3	11.6	5.2	20.1	19.4	....	24.6	32
34	14.3	15.2	12.5	6.1	21.4	20.7	....	26.2	34
36	15.2	16.1	13.4	7.0	22.8	22.0	....	27.9	36
38	16.1	16.9	14.3	7.9	24.2	23.3	....	29.6	38
40	16.9	17.8	15.2	8.8	25.5	24.7	....	31.3	40
42	17.8	18.7	16.1	9.7	26.9	26.0	....	32.9	42
44	18.7	19.6	17.0	10.7	28.3	27.3	....	34.6	44
46	19.6	20.5	17.9	11.6	29.6	28.6	....	36.3	46
48	20.4	21.4	18.8	12.5	31.0	30.0	....	37.9	48
50	21.3	22.3	19.7	13.4	32.3	31.3	....	39.6	50
52	22.2	23.2	20.7	14.3	33.7	32.6	....	41.3	52
54	23.0	24.1	21.6	15.2	35.1	34.0	....	42.9	54
56	23.9	25.0	22.5	16.2	36.4	35.3	....	44.6	56
58	24.8	25.9	23.4	17.1	37.8	36.6	....	46.3	58
60	25.6	26.8	24.3	18.0	39.2	37.9	....	48.0	60
62	26.5	27.7	25.2	18.9	40.5	39.3	....	49.6	62
64	27.4	28.6	26.2	19.8	41.9	40.6	....	51.3	64
66	28.3	29.5	27.1	20.8	43.3	41.9	....	53.0	66
68	29.2	30.4	28.0	21.7	44.7	43.3	40.7	54.6	68
70	30.0	31.3	28.9	22.6	46.0	44.6	41.9	56.3	70
72	30.9	32.3	29.8	23.5	47.4	45.9	43.1	58.0	72
74	31.8	33.2	30.8	24.5	48.8	47.3	44.2	59.6	74
76	32.7	34.1	31.7	25.4	50.1	48.6	45.4	61.3	76
78	33.6	35.0	32.6	26.3	51.5	49.9	46.6	63.0	78
80	34.4	35.9	33.5	27.3	52.9	51.3	47.8	64.6	80
82	35.3	36.8	34.5	28.2	54.2	52.6	49.0	66.3	82
84	36.2	37.7	35.4	29.1	55.6	53.9	50.1	68.0	84
86	37.1	38.6	36.3	30.0	57.0	55.3	51.3	69.7	86
88	38.0	39.5	37.2	31.0	58.4	56.6	52.5	71.3	88

[a] U. S. Bur. Standards Circ. 44, p. 139. The columns headed "Lactose" and "Lactose and Sucrose" were taken from "Methods of Sugar Analysis and Allied Determinations" by Arthur Given.

Munson and Walker table—Continued. **43.011**

(Expressed in mg)

Cuprous oxide (Cu_2O)	Dextrose (*d*-glucose)	Invert sugar	Invert sugar and sucrose		Lactose	Lactose and sucrose		Maltose	Cuprous oxide (Cu_2O)
			0.4 gram total sugar	2 grams total sugar	$C_{12}H_{22}O_{11}+H_2O$	1 lactose, 4 sucrose	1 lactose, 12 sucrose	$C_{12}H_{22}O_{11}+H_2O$	
90	38.9	40.4	38.2	31.9	59.7	57.9	53.7	73.0	90
92	39.8	41.4	39.1	32.8	61.1	59.3	54.9	74.7	92
94	40.6	42.3	40.0	33.8	62.5	60.6	56.0	76.3	94
96	41.5	43.2	41.0	34.7	63.8	61.9	57.2	78.0	96
98	42.4	44.1	41.9	35.6	65.2	63.3	58.4	79.7	98
100	43.3	45.0	42.8	36.6	66.6	64.6	59.6	81.3	100
102	44.2	46.0	43.8	37.5	68.0	66.0	60.8	83.0	102
104	45.1	46.9	44.7	38.5	69.3	67.3	62.0	84.7	104
106	46.0	47.8	45.6	39.4	70.7	68.6	63.2	86.3	106
108	46.9	48.7	46.6	40.3	72.1	70.0	64.4	88.0	108
110	47.8	49.6	47.5	41.3	73.5	71.3	65.6	89.7	110
112	48.7	50.6	48.4	42.2	74.8	72.6	66.7	91.3	112
114	49.6	51.5	49.4	43.2	76.2	74.0	67.9	93.0	114
116	50.5	52.4	50.3	44.1	77.6	75.3	69.1	94.7	116
118	51.4	53.3	51.2	45.0	79.0	76.7	70.3	96.4	118
120	52.3	54.3	52.2	46.0	80.3	78.0	71.5	98.0	120
122	53.2	55.2	53.1	46.9	81.7	79.3	72.7	99.7	122
124	54.1	56.1	54.1	47.9	83.1	80.7	73.9	101.4	124
126	55.0	57.0	55.0	48.8	84.5	82.0	75.1	103.0	126
128	55.9	58.0	55.9	49.8	85.8	83.4	76.3	104.7	128
130	56.8	58.9	56.9	50.7	87.2	84.7	77.5	106.4	130
132	57.7	59.8	57.8	51.7	88.6	86.0	78.7	108.0	132
134	58.6	60.8	58.8	52.6	90.0	87.4	79.7	109.7	134
136	59.5	61.7	59.7	53.6	91.3	88.7	81.1	111.4	136
138	60.4	62.6	60.7	54.5	92.7	90.1	82.3	113.0	138
140	61.3	63.6	61.6	55.5	94.1	91.4	83.5	114.7	140
142	62.2	64.5	62.6	56.4	95.5	92.8	84.7	116.4	142
144	63.1	65.4	63.5	57.4	96.8	94.1	85.9	118.0	144
146	64.0	66.4	64.5	58.3	98.2	95.4	87.1	119.7	146
148	65.0	67.3	65.4	59.3	99.6	96.8	88.3	121.4	148
150	65.9	68.3	66.4	60.2	101.0	98.1	89.5	123.0	150
152	66.8	69.2	67.3	61.2	102.3	99.5	90.8	124.7	152
154	67.7	70.1	68.3	62.1	103.7	100.8	92.0	126.4	154
156	68.6	71.1	69.2	63.1	105.1	102.2	93.2	128.0	156
158	69.5	72.0	70.2	64.1	106.5	103.5	94.4	129.7	158
160	70.4	73.0	71.2	65.0	107.9	104.8	95.6	131.4	160
162	71.4	73.9	72.1	66.0	109.2	106.2	96.8	133.0	162
164	72.3	74.9	73.1	66.9	110.6	107.5	98.0	134.7	164
166	73.2	75.8	74.0	67.9	112.0	108.9	99.2	136.4	166
168	74.1	76.8	75.0	68.8	113.4	110.2	100.4	138.0	168

43.011 *Munson and Walker table*—Continued.
(Expressed in mg)

CUPROUS OXIDE (Cu_2O)	DEXTROSE (*d*-GLUCOSE)	INVERT SUGAR	INVERT SUGAR AND SUCROSE		LACTOSE	LACTOSE AND SUCROSE		MALTOSE	CUPROUS OXIDE (Cu_2O)
			0.4 gram total sugar	2 grams total sugar	$C_{12}H_{22}O_{11}+H_2O$	1 lactose, 4 sucrose	1 lactose, 12 sucrose	$C_{12}H_{22}O_{11}+H_2O$	
170	75.1	77.7	76.0	69.8	114.8	111.6	101.6	139.7	170
172	76.0	78.7	76.9	70.8	116.1	112.9	102.8	141.4	172
174	76.9	79.6	77.9	71.7	117.5	114.3	104.1	143.0	174
176	77.8	80.6	78.8	72.7	118.9	115.6	105.3	144.7	176
178	78.8	81.5	79.8	73.7	120.3	117.0	106.5	146.4	178
180	79.7	82.5	80.8	74.6	121.6	118.3	107.7	148.0	180
182	80.6	83.4	81.7	75.6	123.1	119.7	108.9	149.7	182
184	81.5	84.4	82.7	76.6	124.3	121.0	110.1	151.4	184
186	82.5	85.3	83.7	77.6	125.8	122.4	111.3	153.0	186
188	83.4	86.3	84.6	78.5	127.2	123.7	112.5	154.7	188
190	84.3	87.2	85.6	79.5	128.5	125.1	113.8	156.4	190
192	85.3	88.2	86.6	80.5	129.9	126.4	115.0	158.0	192
194	86.2	89.2	87.6	81.4	131.3	127.8	116.2	159.7	194
196	87.1	90.1	88.5	82.4	132.7	129.2	117.4	161.4	196
198	88.1	91.1	89.5	83.4	134.1	130.5	118.6	163.0	198
200	89.0	92.0	90.5	84.4	135.4	131.9	119.8	164.7	200
202	89.9	93.0	91.4	85.3	136.8	133.2	121.0	166.4	202
204	90.9	94.0	92.4	86.3	138.2	134.6	122.3	168.0	204
206	91.8	94.9	93.4	87.3	139.6	135.9	123.5	169.7	206
208	92.8	95.9	94.4	88.3	141.0	137.3	124.7	171.4	208
210	93.7	96.9	95.4	89.2	142.3	138.6	126.0	173.0	210
212	94.6	97.8	96.3	90.2	143.7	140.0	127.2	174.7	212
214	95.6	98.8	97.3	91.2	145.1	141.4	128.4	176.4	214
216	96.5	99.8	98.3	92.2	146.5	142.7	129.6	178.0	216
218	97.5	100.8	99.3	93.2	147.9	144.1	130.9	179.7	218
220	98.4	101.7	100.3	94.2	149.3	145.4	132.1	181.4	220
222	99.4	102.7	101.2	95.1	150.7	146.8	133.3	183.0	222
224	100.3	103.7	102.2	96.1	152.0	148.1	134.5	184.7	224
226	101.3	104.6	103.2	97.1	153.4	149.5	135.8	186.4	226
228	102.2	105.6	104.2	98.1	154.8	150.8	137.0	188.0	228
230	103.2	106.6	105.2	99.1	156.2	152.2	138.2	189.7	230
232	104.1	107.6	106.2	100.1	157.6	153.6	139.4	191.3	232
234	105.1	108.6	107.2	101.1	159.0	154.9	140.7	193.0	234
236	106.0	109.5	108.2	102.1	160.3	156.3	141.9	194.7	236
238	107.0	110.5	109.2	103.1	161.7	157.6	143.2	196.3	238
240	108.0	111.5	110.1	104.0	163.1	159.0	144.4	198.0	240
242	108.9	112.5	111.1	105.0	164.5	160.3	145.6	199.7	242
244	109.9	113.5	112.1	106.0	165.9	161.7	146.9	201.3	244
246	110.8	114.5	113.1	107.0	167.3	163.1	148.1	203.0	246
248	111.8	115.4	114.1	108.0	168.7	164.4	149.3	204.7	248

Munson and Walker table—Continued. 43.011

(Expressed in mg)

CUPROUS OXIDE (Cu_2O)	DEXTROSE (*d*-GLUCOSE)	INVERT SUGAR	INVERT SUGAR AND SUCROSE		LACTOSE	LACTOSE AND SUCROSE		MALTOSE	CUPROUS OXIDE (Cu_2O)
			0.4 gram total sugar	2 grams total sugar	$C_{12}H_{22}O_{11}+H_2O$	1 lactose, 4 sucrose	1 lactose, 12 sucrose	$C_{12}H_{22}O_{11}+H_2O$	
250	112.8	116.4	115.1	109.0	170.1	165.8	150.6	206.3	250
252	113.7	117.4	116.1	110.0	171.5	167.2	151.8	208.0	252
254	114.7	118.4	117.1	111.0	172.8	168.5	153.1	209.7	254
256	115.7	119.4	118.1	112.0	174.2	169.9	154.3	211.3	256
258	116.6	120.4	119.1	113.0	175.6	171.3	155.5	213.0	258
260	117.6	121.4	120.1	114.0	177.0	172.6	156.8	214.7	260
262	118.6	122.4	121.1	115.0	178.4	174.0	158.0	216.3	262
264	119.5	123.4	122.1	116.0	179.8	175.3	159.3	218.0	264
266	120.5	124.4	123.1	117.0	181.2	176.7	160.5	219.7	266
268	121.5	125.4	124.1	118.0	182.6	178.1	161.8	221.3	268
270	122.5	126.4	125.1	119.0	184.0	179.4	163.0	223.0	270
272	123.4	127.4	126.2	120.0	185.3	180.8	164.3	224.6	272
274	124.4	128.4	127.2	121.1	186.7	182.2	165.5	226.3	274
276	125.4	129.4	128.2	122.1	188.1	183.5	166.8	228.0	276
278	126.4	130.4	129.2	123.1	189.5	184.9	168.0	229.6	278
280	127.3	131.4	130.2	124.1	190.9	186.3	169.3	231.3	280
282	128.3	132.4	131.2	125.1	192.3	187.6	170.5	233.0	282
284	129.3	133.4	132.2	126.1	193.7	189.0	171.8	234.6	284
286	130.3	134.4	133.2	127.1	195.1	190.4	173.0	236.3	286
288	131.3	135.4	134.3	128.1	196.5	191.7	174.3	238.0	288
290	132.3	136.4	135.3	129.2	197.8	193.1	175.5	239.6	290
292	133.2	137.4	136.3	130.2	199.2	194.4	176.8	241.3	292
294	134.2	138.4	137.3	131.2	200.6	195.8	178.1	242.9	294
296	135.2	139.4	138.3	132.2	202.0	197.2	179.3	244.6	296
298	136.2	140.5	139.4	133.2	203.4	198.6	180.6	246.3	298
300	137.2	141.5	140.4	134.2	204.8	199.9	181.8	247.9	300
302	138.2	142.5	141.4	135.3	206.2	201.3	183.1	249.6	302
304	139.2	143.5	142.4	136.3	207.6	202.7	184.4	251.3	304
306	140.2	144.5	143.4	137.3	209.0	204.0	185.6	252.9	306
308	141.2	145.5	144.5	138.3	210.4	205.4	186.9	254.6	308
310	142.2	146.6	145.5	139.4	211.8	206.8	188.1	256.3	310
312	143.2	147.6	146.5	140.4	213.2	208.1	189.4	257.9	312
314	144.2	148.6	147.6	141.4	214.6	209.5	190.7	259.6	314
316	145.2	149.6	148.6	142.4	216.0	210.9	191.9	261.2	316
318	146.2	150.7	149.6	143.5	217.3	212.2	193.2	262.9	318
320	147.2	151.7	150.7	144.5	218.7	213.6	194.4	264.6	320
322	148.2	152.7	151.7	145.5	220.1	215.0	195.7	266.2	322
324	149.2	153.7	152.7	146.6	221.5	216.4	197.0	267.9	324
326	150.2	154.8	153.8	147.6	222.9	217.7	198.2	269.6	326
328	151.2	155.8	154.8	148.6	224.3	219.1	199.5	271.2	328

43.011 *Munson and Walker table*—Continued.
(Expressed in mg)

Cuprous oxide (Cu_2O)	Dextrose (*d*-glucose)	Invert sugar	Invert sugar and sucrose		Lactose	Lactose and sucrose		Maltose	Cuprous oxide (Cu_2O)
			0.4 gram total sugar	2 grams total sugar	$C_{12}H_{22}O_{11}+H_2O$	1 lactose, 4 sucrose	1 lactose, 12 sucrose	$C_{12}H_{22}O_{11}+H_2O$	
330	152.2	156.8	155.8	149.7	225.7	220.5	200.8	272.9	330
332	153.2	157.9	156.9	150.7	227.1	221.8	202.0	274.6	332
334	154.2	158.9	157.9	151.7	228.5	223.2	203.3	276.2	334
336	155.2	159.9	159.0	152.8	229.9	224.6	204.6	277.9	336
338	156.3	161.0	160.0	153.8	231.3	226.0	205.9	279.5	338
340	157.3	162.0	161.0	154.8	232.7	227.4	207.1	281.2	340
342	158.3	163.1	162.1	155.9	234.1	228.7	208.4	282 9	342
344	159.3	164.1	163.1	156.9	235.5	230.1	209.7	284.5	344
346	160.3	165.1	164.2	158.0	236.9	231.5	211.0	286.2	346
348	161.4	166.2	165.2	159.0	238.3	232.9	212.2	287.9	348
350	162.4	167.2	166.3	160.1	239.7	234.3	213.5	289.5	350
352	163.4	168.3	167.3	161.1	241.1	235.6	214.8	291.2	352
354	164.4	169.3	168.4	162.2	242.5	237.0	216.1	292.8	354
356	165.4	170.4	169.4	163.2	243.9	238.4	217.3	294.5	356
358	166.5	171.4	170.5	164.3	245.3	239.8	218.6	296.2	358
360	167.5	172.5	171.5	165.3	246.7	241.2	219.9	297.8	360
362	168.5	173.5	172.6	166.4	248.1	242.5	221.2	299.5	362
364	169.6	174.6	173.7	167.4	249.5	243.9	222.5	301.2	364
366	170.6	175.6	174.7	168.5	250.9	245.3	223.7	302.8	366
368	171.6	176.7	175.8	169.5	252.3	246.7	225.0	304.5	368
370	172.7	177.7	176.8	170.6	253.7	248.1	226.3	306.1	370
372	173.7	178.8	177.9	171.6	255.1	249.5	227.6	307.8	372
374	174.7	179.8	179.0	172.7	256.5	250.9	228.9	309.5	374
376	175.8	180.9	180.0	173.7	257.9	252.2	230.2	311.1	376
378	176.8	182.0	181.1	174.8	259.3	253.6	231.5	312.8	378
380	177.9	183.0	182.1	175.9	260.7	255.0	232.8	314.5	380
382	178.9	184.1	183.2	176.9	262.1	256.4	234.1	316.1	382
384	180.0	185.2	184.3	178.0	263.5	257.8	235.4	317.8	384
386	181.0	186.2	185.4	179.1	264.9	259.2	236.6	319.4	386
388	182.0	187.3	186.4	180.1	266.5	260.5	237.9	321.1	388
390	183.1	188.4	187.5	181.2	267.7	261.9	239.2	322.8	390
392	184.1	189.4	188.6	182.3	269.1	263.3	240.5	324.4	392
394	185.2	190.5	189.7	183.3	270.5	264.7	241.8	326.1	394
396	186.2	191.6	190.7	184.4	271.9	266.1	243.1	327.7	396
398	187.3	192.7	191.8	185.5	273.3	267.5	244.4	329.4	398
400	188.4	193.7	192.9	186.5	274.7	268.9	245.7	331.1	400
402	189.4	194.8	194.0	187.6	276.1	270.3	247.0	332.7	402
404	190.5	195.9	195.0	188.7	277.5	271.7	248.3	334.4	404
406	191.5	197.0	196.1	189.8	278.9	273.0	249.6	336.0	406
408	192.6	198.1	197.2	190.8	280.3	274.4	251.0	337.7	408

Munson and Walker table—Concluded. 43.011

(Expressed in mg)

Cuprous oxide (Cu_2O)	Dextrose (*d*-glucose)	Invert sugar	Invert sugar and sucrose: 0.4 gram total sugar	Invert sugar and sucrose: 2 grams total sugar	Lactose: $C_{12}H_{22}O_{11}+H_2O$	Lactose and sucrose: 1 lactose, 4 sucrose	Lactose and sucrose: 1 lactose, 12 sucrose	Maltose: $C_{12}H_{22}O_{11}+H_2O$	Cuprous oxide (Cu_2O)
410	193.7	199.1	198.3	191.9	281.7	275.8	252.3	339.4	410
412	194.7	200.2	199.4	193.0	283.2	277.2	253.6	341.0	412
414	195.8	201.3	200.5	194.1	284.6	278.6	254.9	342.7	414
416	196.8	202.4	201.6	195.2	286.0	280.0	256.2	344.4	416
418	197.9	203.5	202.6	196.2	287.4	281.4	257.5	346.0	418
420	199.0	204.6	203.7	197.3	288.8	282.8	258.8	347.7	420
422	200.1	205.7	204.8	198.4	290.2	284.2	260.1	349.3	422
424	201.1	206.7	205.9	199.5	291.6	285.6	261.4	351.0	424
426	202.2	207.8	207.0	200.6	293.0	287.0	262.7	352.7	426
428	203.3	208.9	208.1	201.7	294.4	288.4	264.0	354.3	428
430	204.4	210.0	209.2	202.7	295.8	289.8	265.4	356.0	430
432	205.5	211.1	210.3	203.8	297.2	291.2	266.6	357.6	432
434	206.5	212.2	211.4	204.9	298.6	292.6	268.0	359.3	434
436	207.6	213.3	212.5	206.0	300.0	294.0	269.3	361.0	436
438	208.7	214.4	213.6	207.1	301.4	295.4	270.6	362.6	438
440	209.8	215.5	214.7	208.2	302.8	296.8	272.0	364.3	440
442	210.9	216.6	215.8	209.3	304.2	298.2	273.3	365.9	442
444	212.0	217.8	216.9	210.4	305.6	299.6	274.6	367.6	444
446	213.1	218.9	218.0	211.5	307.0	301.0	275.9	369.3	446
448	214.1	220.0	219.1	212.6	308.4	302.4	277.2	370.9	448
450	215.2	221.1	220.2	213.7	309.9	303.8	278.6	372.6	450
452	216.3	222.2	221.4	214.8	311.3	305.2	279.9	374.2	452
454	217.4	223.3	222.5	215.9	312.7	306.6	281.2	375.9	454
456	218.5	224.4	223.6	217.0	314.1	308.0	282.5	377.6	456
458	219.6	225.5	224.7	218.1	315.5	309.4	283.9	379.2	458
460	220.7	226.7	225.8	219.2	316.9	310.8	285.2	380.9	460
462	221.8	227.8	226.9	220.3	318.3	312.2	286.5	382.5	462
464	222.9	228.9	228.1	221.4	319.7	313.6	287.8	384.2	464
466	224.0	230.0	229.2	222.5	321.1	315.0	289.2	385.9	466
468	225.1	231.2	230.3	223.7	322.5	316.4	290.5	387.5	468
470	226.2	232.3	231.4	224.8	323.9	317.7	291.8	389.2	470
472	227.4	233.4	232.5	225.9	325.3	319.1	293.2	390.8	472
474	228.3	234.5	233.7	227.0	326.8	320.5	294.5	392.5	474
476	229.6	235.7	234.8	228.1	328.2	321.9	295.8	394.2	476
478	230.7	236.8	235.9	229.2	329.6	323.3	297.1	395.8	478
480	231.8	237.9	237.1	230.3	331.0	324.7	298.5	397.5	480
482	232.9	239.1	238.2	231.5	332.4	326.1	299.8	399.1	482
484	234.1	240.2	239.3	232.6	333.8	327.5	301.1	400.8	484
486	235.2	241.4	240.5	233.7	335.2	328.9	302.5	402.4	486
488	236.3	242.5	241.6	234.8	336.6	330.3	303.8	404.1	488
490	237.4	243.6	242.7	236.0	338.0	331.7	305.1	405.8	490

43.012 *Revised Hammond table for calcg dextrose, levulose, lactose, invert sugar alone, and invert sugar in presence of sucrose (0.3, 0.4, and 2.0 g of total sugar)*[a]

(Applicable when Cu is detd by analysis)
(Expressed in mg)

COPPER (Cu)	DEXTROSE	LEVULOSE	LACTOSE. H_2O	INVERT SUGAR	INVERT SUGAR AND SUCROSE: 0.3 g of total sugar	INVERT SUGAR AND SUCROSE: 0.4 g of total sugar	INVERT SUGAR AND SUCROSE: 2.0 g of total sugar
10	4.6	5.1	7.7	5.2	3.2	2.9	....
12	5.6	6.1	9.3	6.2	4.2	3.9	....
14	6.5	7.2	10.8	7.2	5.3	4.9	....
16	7.5	8.3	12.3	8.2	6.3	5.9	....
18	8.5	9.3	13.8	9.2	7.3	6.9	....
20	9.4	10.4	15.4	10.2	8.3	7.9	1.9
22	10.4	11.5	16.9	11.2	9.3	8.9	2.9
24	11.4	12.5	18.4	12.3	10.4	10.0	3.9
26	12.3	13.6	19.9	13.3	11.4	11.0	4.9
28	13.3	14.7	21.5	14.3	12.4	12.0	6.0
30	14.3	15.8	23.0	15.3	13.4	13.0	7.0
32	15.3	16.8	24.5	16.3	14.5	14.1	8.0
34	16.2	17.9	26.1	17.3	15.5	15.1	9.0
36	17.2	19.0	27.6	18.3	16.5	16.1	10.1
38	18.2	20.1	29.1	19.4	17.6	17.1	11.1
40	19.2	21.1	30.6	20.4	18.6	18.2	12.1
42	20.1	22.2	32.2	21.4	19.6	19.2	13.1
44	21.1	23.3	33.7	22.4	20.7	20.2	14.2
46	22.1	24.4	35.2	23.5	21.7	21.3	15.2
48	23.1	25.4	36.8	24.5	22.7	22.3	16.2
50	24.1	26.5	38.3	25.5	23.8	23.3	17.3
52	25.1	27.6	39.8	26.5	24.8	24.3	18.3
54	26.1	28.7	41.4	27.6	25.8	25.4	19.3
56	27.0	29.8	42.9	28.6	26.9	26.4	20.4
58	28.0	30.9	44.4	29.6	27.9	27.5	21.4
60	29.0	31.9	46.0	30.6	28.9	28.5	22.5
62	30.0	33.0	47.5	31.7	30.0	29.5	23.5
64	31.0	34.1	49.0	32.7	31.0	30.6	24.5
66	32.0	35.2	50.6	33.7	32.1	31.6	25.6
68	33.0	36.3	52.1	34.8	33.1	32.7	26.6
70	34.0	37.4	53.6	35.8	34.2	33.7	27.7
72	35.0	38.5	55.2	36.8	35.2	34.7	28.7
74	36.0	39.6	56.7	37.9	36.3	35.8	29.8
76	37.0	40.7	58.2	38.9	37.3	36.8	30.8
78	38.0	41.7	59.8	40.0	38.4	37.9	31.9
80	39.0	42.8	61.3	41.0	39.4	38.9	32.9
82	40.0	43.9	62.8	42.0	40.5	40.0	34.0
84	41.0	45.0	64.4	43.1	41.5	41.0	35.0
86	42.0	46.1	65.9	44.1	42.6	42.1	36.1
88	43.0	47.2	67.4	45.2	43.6	43.1	37.1
90	44.0	48.3	69.0	46.2	44.7	44.2	38.2
92	45.0	49.4	70.5	47.3	45.7	45.2	39.2
94	46.0	50.5	72.1	48.3	46.8	46.3	40.3
96	47.0	51.6	73.6	49.4	47.8	47.4	41.3
98	48.0	52.7	75.1	50.4	48.9	48.4	42.4

[a] *J. Research, NBS*, 24, 589 (1940); 41, 211 (1948); *J. Assoc. Offic. Agr. Chemists*, 26, 101 (1943).

Hammond table—Continued. **43.012**

(Expressed in mg)

COPPER (Cu)	DEXTROSE	LEVULOSE	LACTOSE. H_2O	INVERT SUGAR	INVERT SUGAR AND SUCROSE		
					0.3 g of total sugar	0.4 g of total sugar	2.0 g of total sugar
100	49.0	53.8	76.7	51.5	50.0	49.5	43.5
102	50.0	54.9	78.2	52.5	51.0	50.5	44.5
104	51.1	56.0	79.7	53.6	52.1	51.6	45.6
106	52.1	57.1	81.3	54.6	53.1	52.7	46.7
108	53.1	58.2	82.8	55.7	54.2	53.7	47.7
110	54.1	59.3	84.4	56.7	55.3	54.8	48.8
112	55.1	60.4	85.9	57.8	56.3	55.8	49.9
114	56.1	61.6	87.4	58.9	57.4	56.9	50.9
116	57.2	62.7	89.0	59.9	58.5	58.0	52.0
118	58.2	63.8	90.5	61.0	59.5	59.0	53.1
120	59.2	64.9	92.1	62.0	60.6	60.1	54.1
122	60.2	66.0	93.6	63.1	61.7	61.2	55.2
124	61.3	67.1	95.2	64.2	62.8	62.3	56.3
126	62.3	68.2	96.7	65.2	63.8	63.3	57.4
128	63.3	69.3	98.2	66.3	64.9	64.4	58.4
130	64.3	70.4	99.8	67.4	66.0	65.5	59.5
132	65.4	71.6	101.3	68.4	67.1	66.6	60.6
134	66.4	72.7	102.9	69.5	68.1	67.6	61.7
136	67.4	73.8	104.4	70.6	69.2	68.7	62.8
138	68.5	74.9	106.0	71.6	70.3	69.8	63.9
140	69.5	76.0	107.5	72.7	71.4	70.9	64.9
142	70.5	77.1	109.0	73.8	72.5	72.0	66.0
144	71.6	78.3	110.6	74.9	73.5	73.0	67.1
146	72.6	79.4	112.1	75.9	74.6	74.1	68.2
148	73.7	80.5	113.7	77.0	75.7	75.2	69.3
150	74.7	81.6	115.2	78.1	76.8	76.3	70.4
152	75.7	82.8	116.8	79.2	77.9	77.4	71.5
154	76.8	83.9	118.3	80.3	79.0	78.5	72.6
156	77.8	85.0	119.9	81.3	80.1	79.6	73.7
158	78.9	86.1	121.4	82.4	81.2	80.6	74.8
160	79.9	87.3	122.9	83.5	82.2	81.7	75.9
162	81.0	88.4	124.5	84.6	83.3	82.8	77.0
164	82.0	89.5	126.0	85.7	84.4	83.9	78.1
166	83.1	90.6	127.6	86.8	85.5	85.0	79.2
168	84.1	91.8	129.1	87.8	86.6	86.1	80.3
170	85.2	92.9	130.7	88.9	87.7	87.2	81.4
172	86.2	94.0	132.2	90.0	88.8	88.3	82.5
174	87.3	95.2	133.8	91.1	89.9	89.4	83.6
176	88.3	96.3	135.3	92.2	91.0	90.5	84.7
178	89.4	97.4	136.9	93.3	92.1	91.6	85.8
180	90.4	98.6	138.4	94.4	93.2	92.7	86.9
182	91.5	99.7	140.0	95.5	94.3	93.8	88.0
184	92.6	100.9	141.5	96.6	95.4	94.9	89.1
186	93.6	102.0	143.1	97.7	96.5	96.0	90.2
188	94.7	103.1	144.6	98.8	97.6	97.1	91.3

43.012 *Hammond table*—Continued.

(Expressed in mg)

COPPER (Cu)	DEXTROSE	LEVULOSE	LACTOSE. H_2O	INVERT SUGAR	INVERT SUGAR AND SUCROSE		
					0.3 g of total sugar	0.4 g of total sugar	2.0 g of total sugar
190	95.7	104.3	146.2	99.9	98.7	98.2	92.4
192	96.8	105.4	147.7	101.0	99.9	99.4	93.6
194	97.9	106.6	149.3	102.1	101.0	100.5	94.7
196	98.9	107.7	150.8	103.2	102.1	101.6	95.8
198	100.0	108.8	152.4	104.3	103.2	102.7	96.9
200	101.1	110.0	153.9	105.4	104.3	103.8	98.0
202	102.2	111.1	155.5	106.5	105.4	104.9	99.2
204	103.2	112.3	157.0	107.6	106.5	106.0	100.3
206	104.3	113.4	158.6	108.7	107.6	107.2	101.4
208	105.4	114.6	160.2	109.8	108.8	108.3	102.5
210	106.5	115.7	161.7	110.9	109.9	109.4	103.7
212	107.5	116.9	163.3	112.1	111.0	110.5	104.8
214	108.6	118.0	164.8	113.2	112.1	111.6	105.9
216	109.7	119.2	166.4	114.3	113.2	112.8	107.1
218	110.8	120.3	167.9	115.4	114.4	113.9	108.2
220	111.9	121.5	169.5	116.5	115.5	115.0	109.3
222	112.9	122.6	171.0	117.6	116.6	116.1	110.5
224	114.0	123.8	172.6	118.8	117.7	117.3	111.6
226	115.1	125.0	174.2	119.9	118.9	118.4	112.7
228	116.2	126.1	175.7	121.0	120.0	119.5	113.9
230	117.3	127.3	177.3	122.1	121.1	120.7	115.0
232	118.4	128.4	178.8	123.3	122.3	121.8	116.2
234	119.5	129.6	180.4	124.4	123.4	122.9	117.3
236	120.6	130.8	181.9	125.5	124.5	124.1	118.4
238	121.7	131.9	183.5	126.6	125.7	125.2	119.6
240	122.7	133.1	185.1	127.8	126.8	126.3	120.7
242	123.8	134.2	186.6	128.9	127.9	127.5	121.9
244	124.9	135.4	188.2	130.0	129.1	128.6	123.0
246	126.0	136.6	189.7	131.2	130.2	129.8	124.2
248	127.1	137.7	191.3	132.3	131.3	130.9	125.3
250	128.2	138.9	192.9	133.4	132.5	132.0	126.5
252	129.3	140.1	194.4	134.6	133.6	133.2	127.6
254	130.4	141.3	196.0	135.7	134.8	134.3	128.8
256	131.6	142.4	197.5	136.8	135.9	135.5	130.0
258	132.7	143.6	199.1	138.0	137.1	136.6	131.1
260	133.8	144.8	200.7	139.1	138.2	137.8	132.3
262	134.9	145.9	202.2	140.3	139.4	138.9	133.4
264	136.0	147.1	203.8	141.4	140.5	140.1	134.6
266	137.1	148.3	205.3	142.6	141.7	141.2	135.8
268	138.2	149.5	206.9	143.7	142.8	142.4	136.9
270	139.3	150.6	208.5	144.8	144.0	143.5	138.1
272	140.4	151.8	210.0	146.0	145.1	144.7	139.3
274	141.6	153.0	211.6	147.1	146.3	145.9	140.4
276	142.7	154.2	213.2	148.3	147.4	147.0	141.6
278	143.8	155.4	214.7	149.4	148.6	148.2	142.8

Hammond table—Continued. 43.012
(Expressed in mg)

COPPER (Cu)	DEXTROSE	LEVULOSE	LACTOSE. H_2O	INVERT SUGAR	INVERT SUGAR AND SUCROSE 0.3 g of total sugar	0.4 g of total sugar	2.0 g of total sugar
280	144.9	156.5	216.3	150.6	149.7	149.3	143.9
282	146.0	157.7	217.9	151.8	150.9	150.5	145.1
284	147.2	158.9	219.4	152.9	152.1	151.7	146.3
286	148.3	160.1	221.0	154.1	153.2	152.8	147.5
288	149.4	161.3	222.6	155.2	154.4	154.0	148.6
290	150.5	162.5	224.1	156.4	155.5	155.2	149.8
292	151.7	163.7	225.7	157.5	156.7	156.3	151.0
294	152.8	164.9	227.3	158.7	157.9	157.5	152.2
296	153.9	166.0	228.8	159.9	159.0	158.7	153.4
298	155.1	167.2	230.4	161.0	160.2	159.9	154.6
300	156.2	168.4	232.0	162.2	161.4	161.0	155.7
302	157.3	169.6	233.5	163.4	162.5	162.2	156.9
304	158.5	170.8	235.1	164.5	163.7	163.4	158.1
306	159.6	172.0	236.7	165.7	164.9	164.6	159.3
308	160.7	173.2	238.2	166.9	166.1	165.7	160.5
310	161.9	174.4	239.8	168.0	167.2	166.9	161.7
312	163.0	175.6	241.4	169.2	168.4	168.1	162.9
314	164.2	176.8	243.0	170.4	169.6	169.3	164.1
316	165.3	178.0	244.5	171.6	170.8	170.5	165.3
318	166.5	179.2	246.1	172.8	172.0	171.7	166.5
320	167.6	180.4	247.7	173.9	173.1	172.8	167.7
322	168.8	181.6	249.2	175.1	174.3	174.0	168.9
324	169.9	182.8	250.8	176.3	175.5	175.2	170.1
326	171.1	184.0	252.4	177.5	176.7	176.4	171.3
328	172.2	185.2	253.9	178.7	177.9	177.6	172.5
330	173.4	186.4	255.5	179.8	179.1	178.8	173.7
332	174.5	187.6	257.1	181.0	180.3	180.0	174.9
334	175.7	188.8	258.7	182.2	181.5	181.2	176.1
336	176.8	190.1	260.2	183.4	182.6	182.4	177.3
338	178.0	191.3	261.8	184.6	183.8	183.6	178.6
340	179.2	192.5	263.4	185.8	185.0	184.8	179.8
342	180.3	193.7	265.0	187.0	186.2	186.0	181.0
344	181.5	194.9	266.6	188.2	187.4	187.2	182.2
346	182.7	196.1	268.1	189.4	188.6	188.4	183.4
348	183.8	197.3	269.7	190.6	189.8	189.6	184.6
350	185.0	198.5	271.3	191.8	191.0	190.8	185.9
352	186.2	199.8	272.9	193.0	192.2	192.0	187.1
354	187.3	201.0	274.4	194.2	193.4	193.2	188.3
356	188.5	202.2	276.0	195.4	194.6	194.4	189.5
358	189.7	203.4	277.6	196.6	195.8	195.7	190.8
360	190.9	204.7	279.2	197.8	197.1	196.9	192.0
362	192.0	205.9	280.8	199.0	198.3	198.1	193.2
364	193.2	207.1	282.4	200.2	199.5	199.3	194.5
366	194.4	208.3	284.0	201.4	200.7	200.5	195.7
368	195.6	209.6	285.6	202.6	201.9	201.7	196.9

43.012 *Hammond table*—Concluded.

(Expressed in mg)

COPPER (Cu)	DEXTROSE	LEVULOSE	LACTOSE. H_2O	INVERT SUGAR	INVERT SUGAR AND SUCROSE		
					0.3 g of total sugar	0.4 g of total sugar	2.0 g of total sugar
370	196.8	210.8	287.1	203.8	203.1	203.0	198.2
372	198.0	212.0	288.7	205.0	204.3	204.2	199.4
374	199.1	213.3	290.3	206.3	205.6	205.4	200.7
376	200.3	214.5	291.9	207.5	206.8	206.6	201.9
378	201.5	215.7	293.5	208.7	208.0	207.9	203.1
380	202.7	217.0	295.0	209.9	209.2	209.1	204.4
382	203.9	218.2	296.6	211.1	210.4	210.3	205.6
384	205.1	219.5	298.2	212.4	211.7	211.6	206.9
386	206.3	220.7	299.8	213.6	212.9	212.8	208.1
388	207.5	221.9	301.4	214.8	214.1	214.0	209.4
390	208.7	223.2	303.0	216.0	215.4	215.3	210.6
392	209.9	224.4	304.6	217.3	216.6	216.5	211.9
394	211.1	225.7	306.2	218.5	217.8	217.8	213.2
396	212.3	226.9	307.8	219.8	219.1	219.0	214.4
398	213.5	228.2	309.4	221.0	220.3	220.3	215.7
400	214.7	229.4	311.0	222.2	221.5	221.5	217.0
402	215.9	230.7	312.6	223.5	222.8	222.8	218.2
404	217.1	232.0	314.2	224.7	224.0	224.0	219.5
406	218.4	233.2	315.9	226.0	225.3	225.3	220.8
408	219.6	234.5	317.5	227.2	226.6	226.5	222.0
410	220.8	235.8	319.1	228.5	227.8	227.8	223.3
412	222.0	237.1	320.7	229.7	229.1	229.1	224.6
414	223.3	238.4	322.4	231.0	230.4	230.4	225.9
416	224.5	239.7	324.0	232.3	231.6	231.7	227.2
418	225.7	241.0	325.7	233.6	232.9	232.9	228.5
420	227.0	242.2	327.4	234.8	234.2	234.2	229.8
422	228.2	243.6	329.1	236.1	235.5	235.5	231.1
424	229.5	244.9	330.8	237.5	236.8	236.9	232.4
426	230.7	246.3	332.6	238.8	238.2	238.2	233.8
428	232.0	247.8	334.4	240.2	239.5	239.6	235.1
430	233.3	249.2	336.3	241.5	240.9	241.0	236.5
432	234.7	250.8	338.3	243.0	242.4	242.5	238.0
434	236.1	252.7	340.7	244.7	244.1	244.2	239.6

Corrections to be applied to iodine titer to obtain mg of invert sugar by Ofner method[a] **43.013**

0.0323 N iodine soln (ml)	Weight of sucrose (grams)									
	1	2	3	4	5	6	7	8	9	10
1	0.11	0.22	0.34	0.45	0.55	0.66	0.77	0.89	1.00	1.11
2	.17	.28	.40	.51	.61	.72	.84	.95	1.06	1.16
3	.22	.34	.45	.57	.67	.78	.90	1.01	1.12	1.22
4	.28	.39	.51	.62	.73	.84	.95	1.07	1.18	1.28
5	.33	.45	.56	.68	.78	.90	1.01	1.12	1.24	1.33
6	.39	.50	.61	.73	.83	.95	1.06	1.18	1.29	1.39
7	.44	.55	.67	.78	.88	1.00	1.11	1.23	1.34	1.44
8	.49	.60	.72	.83	.94	1.05	1.16	1.28	1.39	1.50
9	.54	.65	.76	.88	.99	1.10	1.21	1.33	1.44	1.55
10	.59	.70	.82	.93	1.03	1.15	1.26	1.37	1.49	1.60
11	.63	.75	.86	.98	1.08	1.20	1.31	1.42	1.54	1.65
12	.67	.78	.90	1.02	1.12	1.24	1.35	1.47	1.58	1.69
13	.70	.82	.93	1.05	1.16	1.27	1.39	1.51	1.62	1.72
14	.74	.85	.97	1.09	1.19	1.31	1.42	1.54	1.65	1.76
15	.77	.88	1.00	1.12	1.22	1.34	1.45	1.57	1.69	1.79
16	.80	.91	1.03	1.15	1.25	1.37	1.48	1.60	1.72	1.82
17	.82	.94	1.05	1.18	1.28	1.40	1.51	1.63	1.74	1.85
18	.84	.96	1.08	1.20	1.30	1.42	1.54	1.66	1.77	1.88
19	.86	.98	1.10	1.22	1.32	1.45	1.56	1.68	1.79	1.90
20	.88	1.00	1.11	1.24	1.34	1.46	1.58	1.70	1.81	1.92
21	.89	1.01	1.13	1.25	1.35	1.48	1.59	1.71	1.83	1.94
22	.86	.98	1.11	1.23	1.34	1.47	1.59	1.71	1.84	1.95

[a] *J. Assoc. Offic. Agr. Chemists*, 26, 470 (1943).

Meissl and Hiller factors for detg invert sugar in materials in which, of total sugars present, >1.5% is invert sugar, and <98.5% is sucrose[a] **43.014**

Ratio of sucrose to invert sugar = R:1	Approximate absolute weight of invert sugar (Z)						
	200 mg	175 mg	150 mg	125 mg	100 mg	75 mg	50 mg
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
0:100	56.4	55.4	54.5	53.8	53.2	53.0	53.0
10:90	56.3	55.3	54.4	53.8	53.2	52.9	52.9
20:80	56.2	55.2	54.3	53.7	53.2	52.7	52.7
30:70	56.1	55.1	54.2	53.7	53.2	52.6	52.6
40:60	55.9	55.0	54.1	53.6	53.1	52.5	52.4
50:50	55.7	54.9	54.0	53.5	53.1	52.3	52.2
60:40	55.6	54.7	53.8	53.2	52.8	52.1	51.9
70:30	55.5	54.5	53.5	52.9	52.5	51.9	51.6
80:20	55.4	54.3	53.3	52.7	52.2	51.7	51.3
90:10	54.6	53.6	53.1	52.6	52.1	51.6	51.2
91:9	54.1	53.6	52.6	52.1	51.6	51.2	50.7
92:8	53.6	53.1	52.1	51.6	51.2	50.7	50.3
93:7	53.6	53.1	52.1	51.2	50.7	50.3	49.8
94:6	53.1	52.6	51.6	50.7	50.3	49.8	48.9
95:5	52.6	52.1	51.2	50.3	49.4	48.9	48.5
96:4	52.1	51.2	50.7	49.8	48.9	47.7	46.9
97:3	50.7	50.3	49.8	48.9	47.7	46.2	45.1
98:2	49.9	48.9	48.5	47.3	45.8	43.3	40.0
99:1	47.7	47.3	46.5	45.1	43.3	41.2	38.1

[a] *Z. Ver. Ruebenzucker-Ind.*, **39** (N.F. 26) 734 (1889).

43.015 *Wein table for detn of maltose*[a]

(Expressed in mg)

COPPER	CUPROUS OXIDE	MALTOSE	COPPER	CUPROUS OXIDE	MALTOSE	COPPER	CUPROUS OXIDE	MALTOSE
32	36.0	27.0	122	137.4	106.2	212	238.7	186.8
34	38.3	28.7	124	139.6	108.0	214	240.9	188.6
36	40.5	30.5	126	141.9	109.8	216	243.2	190.4
38	42.8	32.2	128	144.1	111.6	218	245.4	192.1
40	45.0	33.9	130	146.4	113.4	220	247.7	193.9
42	47.3	35.7	132	148.6	115.2	222	249.9	195.7
44	49.5	37.4	134	150.9	117.0	224	252.4	197.5
46	51.8	39.1	136	153.1	118.8	226	254.4	199.3
48	54.0	40.9	138	155.4	120.6	228	256.7	201.1
50	56.3	42.6	140	157.6	122.4	230	258.9	202.9
52	58.5	44.4	142	159.9	124.2	232	261.2	204.7
54	60.8	46.1	144	162.1	126.0	234	263.4	206.5
56	63.0	47.8	146	164.4	127.8	236	265.7	208.3
58	65.3	49.6	148	166.6	129.6	238	268.0	210.0
60	67.6	51.3	150	168.9	131.4	240	270.2	211.8
62	69.8	53.1	152	171.1	133.2	242	272.5	213.6
64	72.1	54.8	154	173.4	135.0	244	274.7	215.4
66	74.3	56.6	156	175.6	136.8	246	277.0	217.2
68	76.6	58.3	158	177.9	138.6	248	279.2	219.0
70	78.8	60.1	160	180.1	140.4	250	281.5	220.8
72	81.1	61.8	162	182.4	142.2	252	283.7	222.6
74	83.3	63.6	164	184.6	144.0	254	286.0	224.4
76	85.6	65.4	166	186.9	145.8	256	288.2	226.2
78	87.8	67.1	168	189.1	147.8	258	290.5	228.0
80	90.1	68.9	170	191.4	149.4	260	292.7	229.8
82	92.3	70.6	172	193.6	151.2	262	295.0	231.6
84	94.6	72.4	174	195.9	152.9	264	297.2	233.4
86	96.8	74.1	176	198.1	154.7	266	299.5	235.2
88	99.1	75.9	178	200.4	156.5	268	301.7	237.0
90	101.3	77.7	180	202.6	158.3	270	304.0	238.8
92	103.6	79.5	182	204.9	160.1	272	306.2	240.6
94	105.8	81.2	184	207.1	161.8	274	308.5	242.4
96	108.1	83.0	186	209.4	163.6	276	310.7	244.2
98	110.3	84.8	188	211.7	165.4	278	313.0	246.0
100	112.6	86.6	190	213.9	167.2	280	315.2	247.8
102	114.8	88.4	192	216.2	169.0	282	317.5	249.6
104	117.1	90.1	194	218.4	170.7	284	319.7	251.3
106	119.3	91.9	196	220.7	172.5	286	322.0	253.1
108	121.6	93.7	198	222.9	174.3	288	324.2	254.9
110	123.8	95.5	200	225.2	176.1	290	326.5	256.6
112	126.1	97.3	202	227.4	177.9	292	328.7	258.4
114	128.3	99.0	204	229.7	179.6	294	331.0	260.2
116	130.6	100.8	206	231.9	181.4	296	333.2	262.0
118	132.8	102.6	208	234.2	183.2	298	335.5	263.7
120	135.1	104.4	210	236.4	185.0	300	337.8	265.5

[a] Tables for Quantitative Estimation of Sugars. Translated by Frew, 1896, p. 26.

Copper-levulose equivalents according to Jackson and Mathews modification of Nyns selective method for levulose 43.016

(Expressed in mg. Linear interpolation yields accurate results)

Cu	LEVULOSE	Cu	LEVULOSE
5	2.5	130	39.3
10	4.5	140	42.0
15	6.2	150	44.7
20	7.9	160	47.4
25	9.5	170	50.0
30	11.0	180	52.6
35	12.5	190	55.2
40	13.9	200	57.9
45	15.4	210	60.6
50	16.8	220	63.4
55	18.3	230	66.4
60	19.7	240	69.4
65	21.2	250	72.5
70	22.5	260	75.7
80	25.4	270	79.0
90	28.1	280	82.4
100	30.9	290	85.9
110	33.7	300	89.5
120	36.5	310	93.2

43.017 *Total reducing sugar required for complete reduction of 10 ml Soxhlet soln to be used in connection with Lane-Eynon general volumetric method*

TITER	INVERT SUGAR NO SUCROSE	1 GRAM SUCROSE PER 100 ML INVERT SUGAR	5 GRAMS SUCROSE PER 100 ML INVERT SUGAR	10 GRAMS SUCROSE PER 100 ML INVERT SUGAR	25 GRAMS SUCROSE PER 100 ML INVERT SUGAR	DEXTROSE	LEVULOSE	ANHYDROUS MALTOSE $C_{12}H_{22}O_{11}$	HYDRATED MALTOSE $C_{12}H_{22}O_{11} \cdot H_2O$	ANHYDROUS LACTOSE $C_{12}H_{22}O_{11}$	HYDRATED LACTOSE $C_{12}H_{22}O_{11} \cdot H_2O$
15	50.5	49.9	47.6	46.1	43.4	49.1	52.2	77.2	81.3	64.9	68.3
16	50.6	50.0	47.6	46.1	43.4	49.2	52.3	77.1	81.2	64.8	68.2
17	50.7	50.1	47.6	46.1	43.4	49.3	52.3	77.0	81.1	64.8	68.2
18	50.8	50.1	47.6	46.1	43.3	49.3	52.4	77.0	81.0	64.7	68.1
19	50.8	50.2	47.6	46.1	43.3	49.4	52.5	76.9	80.9	64.7	68.1
20	50.9	50.2	47.6	46.1	43.2	49.5	52.5	76.8	80.8	64.6	68.0
21	51.0	50.2	47.6	46.1	43.2	49.5	52.6	76.7	80.7	64.6	68.0
22	51.0	50.3	47.6	46.1	43.1	49.6	52.7	76.6	80.6	64.6	68.0
23	51.1	50.3	47.6	46.1	43.0	49.7	52.7	76.5	80.5	64.5	67.9
24	51.2	50.3	47.6	46.1	42.9	49.8	52.8	76.4	80.4	64.5	67.9
25	51.2	50.4	47.6	46.0	42.8	49.8	52.8	76.4	80.4	64.5	67.9
26	51.3	50.4	47.6	46.0	42.8	49.9	52.9	76.3	80.3	64.5	67.9
27	51.4	50.4	47.6	46.0	42.7	49.9	52.9	76.2	80.2	64.4	67.8
28	51.4	50.5	47.7	46.0	42.7	50.0	53.0	76.1	80.1	64.4	67.8
29	51.5	50.5	47.7	46.0	42.6	50.0	53.1	76.0	80.0	64.4	67.8
30	51.5	50.5	47.7	46.0	42.5	50.1	53.2	76.0	80.0	64.4	67.8
31	51.6	50.6	47.7	45.9	42.5	50.2	53.2	75.9	79.9	64.4	67.8
32	51.6	50.6	47.7	45.9	42.4	50.2	53.3	75.9	79.9	64.4	67.8
33	51.7	50.6	47.7	45.9	42.3	50.3	53.3	75.8	79.8	64.4	67.8
34	51.7	50.6	47.7	45.8	42.2	50.3	53.4	75.8	79.8	64.4	67.9
35	51.8	50.7	47.7	45.8	42.2	50.4	53.4	75.7	79.7	64.5	67.9
36	51.8	50.7	47.7	45.8	42.1	50.4	53.5	75.6	79.6	64.5	67.9
37	51.9	50.7	47.7	45.7	42.0	50.5	53.5	75.6	79.6	64.5	67.9
38	51.9	50.7	47.7	45.7	42.0	50.5	53.6	75.5	79.5	64.5	67.9
39	52.0	50.8	47.7	45.7	41.9	50.6	53.6	75.5	79.5	64.5	67.9
40	52.0	50.8	47.7	45.6	41.8	50.6	53.6	75.4	79.4	64.5	67.9
41	52.1	50.8	47.7	45.6	41.8	50.7	53.7	75.4	79.4	64.6	68.0
42	52.1	50.8	47.7	45.6	41.7	50.7	53.7	75.3	79.3	64.6	68.0
43	52.2	50.8	47.7	45.5	41.6	50.8	53.8	75.3	79.3	64.6	68.0
44	52.2	50.9	47.7	45.5	41.5	50.8	53.8	75.2	79.2	64.6	68.0
45	52.3	50.9	47.7	45.4	41.4	50.9	53.9	75.2	79.2	64.7	68.1
46	52.3	50.9	47.7	45.4	41.4	50.9	53.9	75.1	79.1	64.7	68.1
47	52.4	50.9	47.7	45.3	41.3	51.0	53.9	75.1	79.1	64.8	68.2
48	52.4	50.9	47.7	45.3	41.2	51.0	54.0	75.1	79.1	64.8	68.2
49	52.5	51.0	47.7	45.2	41.1	51.0	54.0	75.0	79.0	64.8	68.2
50	52.5	51.0	47.7	45.2	41.0	51.1	54.0	75.0	79.0	64.9	68.3

Total reducing sugar required for complete reduction of 25 ml Soxhlet soln to be used in connection with Lane-Eynon general volumetric method **43.018**

TITER	INVERT SUGAR NO SUCROSE	1 GRAM SUCROSE PER 100 ML INVERT SUGAR	DEXTROSE	LEVULOSE	ANHYDROUS MALTOSE $C_{12}H_{22}O_{11}$	HYDRATED MALTOSE $C_{12}H_{22}O_{11}\cdot H_2O$	ANHYDROUS LACTOSE $C_{12}H_{22}O_{11}$	HYDRATED LACTOSE $C_{12}H_{22}O_{11}\cdot H_2O$
15	123.6	122.6	120.2	127.4	197.8	208.2	163.9	172.5
16	123.6	122.7	120.2	127.4	197.4	207.8	163.5	172.1
17	123.6	122.7	120.2	127.5	197.0	207.4	163.1	171.7
18	123.7	122.7	120.2	127.5	196.7	207.1	162.8	171.4
19	123.7	122.8	120.3	127.6	196.5	206.8	162.5	171.1
20	123.8	122.8	120.3	127.6	196.2	206.5	162.3	170.9
21	123.8	122.8	120.3	127.7	195.8	206.1	162.0	170.6
22	123.9	122.9	120.4	127.7	195.5	205.8	161.8	170.4
23	123.9	122.9	120.4	127.8	195.1	205.4	161.6	170.2
24	124.0	122.9	120.5	127.8	194.8	205.1	161.5	170.0
25	124.0	123.0	120.5	127.9	194.5	204.8	161.4	169.9
26	124.1	123.0	120.6	127.9	194.2	204.4	161.2	169.7
27	124.1	123.0	120.6	128.0	193.9	204.1	161.0	169.5
28	124.2	123.1	120.7	128.0	193.6	203.8	160.8	169.3
29	124.2	123.1	120.7	128.1	193.3	203.5	160.7	169.2
30	124.3	123.1	120.8	128.1	193.0	203.2	160.6	169.0
31	124.3	123.2	120.8	128.1	192.8	202.9	160.5	168.9
32	124.4	123.2	120.8	128.2	192.5	202.6	160.4	168.8
33	124.4	123.2	120.9	128.2	192.2	202.3	160.2	168.6
34	124.5	123.3	120.9	128.3	191.9	202.0	160.1	168.5
35	124.5	123.3	121.0	128.3	191.7	201.8	160.0	168.4
36	124.6	123.3	121.0	128.4	191.4	201.5	159.8	168.2
37	124.6	123.4	121.1	128.4	191.2	201.2	159.7	168.1
38	124.7	123.4	121.2	128.5	191.0	201.0	159.6	168.0
39	124.7	123.4	121.2	128.5	190.8	200.8	159.5	167.9
40	124.8	123.4	121.2	128.6	190.5	200.5	159.4	167.8
41	124.8	123.5	121.3	128.6	190.3	200.3	159.3	167.7
42	124.9	123.5	121.4	128.6	190.1	200.1	159.2	167.6
43	124.9	123.5	121.4	128.7	189.8	199.8	159.2	167.6
44	125.0	123.6	121.5	128.7	189.6	199.6	159.1	167.5
45	125.0	123.6	121.5	128.8	189.4	199.4	159.0	167.4
46	125.1	123.6	121.6	128.8	189.2	199.2	159.0	167.4
47	125.1	123.7	121.6	128.9	189.0	199.0	158.9	167.3
48	125.2	123.7	121.7	128.9	188.9	198.9	158.8	167.2
49	125.2	123.7	121.7	129.0	188.8	198.7	158.8	167.2
50	125.3	123.8	121.8	129.0	188.7	198.6	158.7	167.1

43.019 *Quisumbing and Thomas table for calcg dextrose, levulose, invert sugar, lactose, and maltose*

(Expressed in mg)

Copper (Cu)	Cuprous oxide (Cu_2O)	Dextrose (*d*-glucose)	Levulose (*d*-fructose)	Invert sugar	Lactose		Maltose	
					$C_{12}H_{22}O_{11}$	$C_{12}H_{22}O_{11}.H_2O$	$C_{12}H_{22}O_{11}$	$C_{12}H_{22}O_{11}.H_2O$
10	11.1	4.8	5.3	5.0	7.7	8.1	9.4	9.9
20	22.5	9.5	10.5	10.1	15.5	16.3	18.8	19.8
30	33.8	14.3	15.8	15.2	23.2	24.4	28.2	29.7
40	45.0	19.1	21.2	20.3	30.9	32.5	37.6	39.6
50	56.3	24.0	26.5	25.4	38.7	40.7	47.0	49.5
60	67.6	28.9	31.9	30.6	46.4	48.8	56.4	59.4
70	78.8	33.7	37.2	35.7	54.0	56.9	65.8	69.3
80	90.1	38.7	42.6	40.9	61.7	65.0	75.2	79.2
90	101.3	43.6	48.0	46.1	69.5	73.2	84.6	89.1
100	112.6	48.6	53.4	51.3	77.2	81.3	94.0	99.0
110	123.8	53.5	58.8	56.5	85.0	89.5	103.4	108.9
120	135.1	58.5	64.3	61.8	92.7	97.6	112.8	118.8
130	146.4	63.5	69.7	67.0	100.4	105.7	122.2	128.7
140	157.6	68.6	75.2	72.3	108.2	113.9	131.6	138.6
150	168.9	73.7	80.7	77.6	116.0	122.0	141.0	148.5
160	180.1	78.8	86.2	82.9	123.7	130.1	150.4	158.4
170	191.4	83.9	91.7	88.3	131.4	138.3	159.8	168.3
180	202.6	89.1	97.2	93.7	139.1	146.4	169.2	178.2
190	213.9	94.2	102.8	99.1	146.9	154.6	178.8	188.1
200	225.2	99.4	108.4	104.4	154.6	162.7	188.2	198.0
210	236.4	104.6	114.0	109.8	162.3	170.9	197.6	207.9
220	247.7	109.9	119.6	115.2	170.0	179.0	207.0	217.8
230	258.9	115.1	125.2	120.6	177.8	187.2	216.4	227.7
240	270.2	120.4	130.8	126.1	185.5	195.3	225.8	237.6
250	281.5	125.7	136.4	131.6	193.2	203.4	235.2	247.5
260	292.7	131.0	142.1	137.1	201.0	211.6	244.6	257.4
270	304.0	136.4	147.8	142.6	208.8	219.8	254.0	267.3
280	315.2	141.7	153.5	148.2	216.5	227.9	263.4	277.2
290	326.5	147.1	159.2	153.7	224.2	236.0	272.8	287.1
300	337.8	152.6	165.0	159.3	232.0	244.2	282.2	297.0
310	349.0	158.0	170.7	164.9	239.7	252.3	291.6	306.9
320	360.3	163.5	176.5	170.5	247.5	260.5	301.0	316.8
330	371.5	168.9	182.3	176.1	255.3	268.7	310.4	326.7
340	382.8	174.5	188.1	181.8	263.0	276.8	319.8	336.6
350	394.0	180.0	193.9	187.4	270.7	285.0	329.2	346.5
360	405.3	185.5	199.7	193.1	278.4	293.1	338.6	356.4
370	416.6	191.1	205.5	198.8	286.2	301.3	348.0	366.3
380	427.8	196.7	211.4	204.5	293.9	309.4	357.4	376.2
390	439.1	202.3	217.3	210.2	301.6	317.5	366.8	386.1
400	450.3	208.0	223.2	216.0	309.4	325.7	376.2	396.0
410	461.6	213.7	229.1	221.8	317.1	333.8	385.6	405.9
420	472.9	219.4	235.0	227.6	324.9	342.0	395.0	415.8
430	484.1	225.1	240.9	233.4	332.6	350.1	404.4	425.7
440	495.4	230.8	246.9	239.2	340.4	358.3	413.8	435.6
450	506.6	236.6	252.9	245.0	348.1	366.4	423.2	445.5
460	517.9	242.4	258.9	250.9	355.9	374.6	432.6	455.4
470	529.1	248.1	264.9	256.8	363.6	382.7	442.0	465.3
480	540.4	254.8	270.9	262.7	371.3	390.9	451.4	475.2

Kröber table for detn of pentoses and pentosans 43.020

(Expressed in g)

FURFURAL PHLOROGLUCIDE	FURFURAL	ARABINOSE	ARABAN	XYLOSE	XYLAN	PENTOSE	PENTOSAN
0.030	0.0182	0.0391	0.0344	0.0324	0.0285	0.0358	0.0315
.032	.0193	.0413	.0363	.0342	.0301	.0378	.0333
.034	.0203	.0435	.0383	.0361	.0317	.0398	.0350
.036	.0214	.0457	.0402	.0379	.0334	.0418	.0368
.038	.0224	.0479	.0422	.0398	.0350	.0439	.0386
.040	.0235	.0501	.0441	.0416	.0366	.0459	.0404
.042	.0245	.0523	.0460	.0434	.0382	.0479	.0422
.044	.0255	.0545	.0480	.0452	.0398	.0499	.0440
.046	.0266	.0567	.0499	.0471	.0414	.0519	.0457
.048	.0276	.0589	.0519	.0489	.0430	.0539	.0475
.050	.0286	.0611	.0538	.0507	.0446	.0559	.0492
.052	.0297	.0633	.0557	.0525	.0462	.0579	.0510
.054	.0307	.0655	.0576	.0543	.0478	.0599	.0528
.056	.0318	.0677	.0596	.0562	.0494	.0620	.0546
.058	.0328	.0699	.0615	.0580	.0510	.0640	.0564
.060	.0338	.0721	.0634	.0598	.0526	.0660	.0581
.062	.0349	.0743	.0653	.0616	.0542	.0680	.0599
.064	.0359	.0765	.0673	.0635	.0558	.0700	.0617
.066	.0370	.0787	.0692	.0653	.0575	.0720	.0634
.068	.0380	.0809	.0712	.0672	.0591	.0741	.0652
.070	.0390	.0831	.0731	.0690	.0607	.0761	.0670
.072	.0401	.0853	.0750	.0708	.0623	.0781	.0688
.074	.0411	.0875	.0770	.0726	.0639	.0801	.0706
.076	.0422	.0897	.0789	.0745	.0655	.0821	.0722
.078	.0432	.0919	.0809	.0763	.0671	.0841	.0740
.080	.0442	.0941	.0828	.0781	.0687	.0861	.0758
082	.0453	.0963	.0847	.0799	.0703	.0881	.0776
.084	.0463	.0985	.0867	.0817	.0719	.0901	.0794
086	.0474	.1007	.0886	.0836	.0735	.0922	.0812
088	.0484	.1029	.0906	.0854	.0751	.0942	.0830
.090	.0494	.1051	.0925	.0872	.0767	.0962	.0847
.092	.0505	.1073	.0944	.0890	.0783	.0982	.0865
.094	.0515	.1095	.0964	.0909	.0800	.1002	.0883
.096	.0525	.1117	.0983	.0927	.0816	.1022	.0899
.098	.0536	.1139	.1003	.0946	.0832	.1043	.0917
.100	.0546	.1161	.1022	.0964	.0848	.1063	.0935
.102	.0557	.1182	.1041	.0982	.0864	.1083	.0953
.104	.0567	.1204	.1060	.1000	.0880	.1103	.0971
.106	.0577	.1226	.1080	.1019	.0896	.1123	.0988
.108	.0588	.1248	.1099	.1037	.0912	.1143	.1006
.110	.0598	.1270	.1118	.1055	.0928	.1163	.1023
.112	.0608	.1292	.1137	.1073	.0944	.1183	.1041
.114	.0619	.1314	.1156	.1091	.0960	.1203	.1059
.116	.0629	.1336	.1176	.1110	.0976	.1223	.1076
.118	.0640	.1358	.1195	.1128	.0992	.1243	.1094

43.020 *Kröber table*—Continued.

(Expressed in g)

FURFURAL PHLOROGLUCIDE	FURFURAL	ARABINOSE	ARABAN	XYLOSE	XYLAN	PENTOSE	PENTOSAN
0.120	0.0650	0.1380	0.1214	0.1146	0.1008	0.1263	0.1111
.122	.0660	.1402	.1233	.1164	.1024	.1283	.1129
.124	.0671	.1424	.1253	.1182	.1040	.1303	.1147
.126	.0681	.1446	.1272	.1201	.1057	.1324	.1165
.128	.0691	.1468	.1292	.1219	.1073	.1344	.1183
.130	.0702	.1490	.1311	.1237	.1089	.1364	.1201
.132	.0712	.1512	.1330	.1255	.1105	.1384	.1219
.134	.0723	.1534	.1350	.1273	.1121	.1404	.1236
.136	.0733	.1556	.1369	.1292	.1137	.1424	.1253
.138	.0743	.1578	.1389	.1310	.1153	.1444	.1271
.140	.0754	.1600	.1408	.1328	.1169	.1464	.1288
.142	.0764	.1622	.1427	.1346	.1185	.1484	.1306
.144	.0774	.1644	.1447	.1364	.1201	.1504	.1324
.146	.0785	.1666	.1466	.1383	.1217	.1525	.1342
.148	.0795	.1688	.1486	.1401	.1233	.1545	.1360
.150	.0805	.1710	.1505	.1419	.1249	.1565	.1377
.152	.0816	.1732	.1524	.1437	.1265	.1585	.1395
.154	.0826	.1754	.1544	.1455	.1281	.1605	.1413
.156	.0837	.1776	.1563	.1474	.1297	.1625	.1430
.158	.0847	.1798	.1583	.1492	.1313	.1645	.1448
.160	.0857	.1820	.1602	.1510	.1329	.1665	.1465
.162	.0868	.1842	.1621	.1528	.1345	.1685	.1483
.164	.0878	.1864	.1640	.1546	.1361	.1705	.1501
.166	.0888	.1886	.1660	.1565	.1377	.1726	.1519
.168	.0899	.1908	.1679	.1583	.1393	.1746	.1537
.170	.0909	.1930	.1698	.1601	.1409	.1766	.1554
.172	.0920	.1952	.1717	.1619	.1425	.1786	.1572
.174	.0930	.1974	.1736	.1637	.1441	.1806	.1590
.176	.0940	.1996	.1756	.1656	.1457	.1826	.1607
.178	.0951	.2018	.1775	.1674	.1473	.1846	.1625
.180	.0961	.2039	.1794	.1692	.1489	.1866	.1642
.182	.0971	.2061	.1813	.1710	.1505	.1886	.1660
.184	.0982	.2082	.1832	.1728	.1521	.1906	.1678
.186	.0992	.2104	.1851	.1747	.1537	.1926	.1695
.188	.1003	.2126	.1870	.1765	.1553	.1946	.1712
.190	.1013	.2147	.1889	.1783	.1569	.1965	.1729
.192	.1023	.2168	.1908	.1801	.1585	.1985	.1747
.194	.1034	.2190	.1927	.1819	.1601	.2005	.1764
.196	.1044	.2212	.1946	.1838	.1617	.2025	.1782
.198	.1054	.2233	.1965	.1856	.1633	.2045	.1800
.200	.1065	.2255	.1984	.1874	.1649	.2065	.1817
.202	.1075	.2276	.2003	.1892	.1665	.2085	.1835
.204	.1085	.2298	.2022	.1910	.1681	.2105	.1853
.206	.1096	.2320	.2041	.1929	.1697	.2125	.1869
.208	.1106	.2341	.2060	.1947	.1713	.2144	.1887

Kröber table—Concluded. 43.020
(Expressed in g)

FURFURAL PHLOROGLUCIDE	FURFURAL	ARABINOSE	ARABAN	XYLOSE	XYLAN	PENTOSE	PENTOSAN
0.210	0.1116	0.2363	0.2079	0.1965	0.1729	0.2164	0.1904
.212	.1127	.2384	.2098	.1984	.1745	.2184	.1922
.214	.1137	.2406	.2117	.2002	.1761	.2204	.1940
.216	.1147	.2428	.2136	.2020	.1778	.2224	.1957
.218	.1158	.2449	.2155	.2038	.1794	.2244	.1974
.220	.1168	.2471	.2174	.2057	.1810	.2264	.1992
.222	.1178	.2492	.2193	.2075	.1826	.2284	.2010
.224	.1189	.2514	.2212	.2093	.1842	.2304	.2028
.226	.1199	.2536	.2232	.2111	.1858	.2324	.2046
.228	.1209	.2557	.2251	.2130	.1874	.2344	.2063
.230	.1220	.2579	.2270	.2148	.1890	.2364	.2081
.232	.1230	.2600	.2289	.2166	.1906	.2383	.2097
.234	.1240	.2622	.2308	.2184	.1922	.2403	.2115
.236	.1251	.2644	.2327	.2202	.1938	.2423	.2132
.238	.1261	.2665	.2346	.2220	.1954	.2443	.2150
.240	.1271	.2687	.2365	.2239	.1970	.2463	.2168
.242	.1281	.2708	.2384	.2257	.1986	.2483	.2185
.244	.1292	.2730	.2403	.2275	.2002	.2503	.2203
.246	.1302	.2752	.2422	.2293	.2018	.2523	.2220
.248	.1312	.2773	.2441	.2311	.2034	.2543	.2238
.250	.1323	.2795	.2460	.2330	.2050	.2563	.2256
.252	.1333	.2816	.2479	.2348	.2066	.2582	.2272
.254	.1343	.2838	.2498	.2366	.2082	.2602	.2290
.256	.1354	.2860	.2517	.2384	.2098	.2622	.2307
.258	.1364	.2881	.2536	.2402	.2114	.2642	.2325
.260	.1374	.2903	.2555	.2420	.2130	.2662	.2342
.262	.1385	.2924	.2574	.2438	.2146	.2681	.2359
.264	.1395	.2946	.2593	.2456	.2162	.2701	.2377
.266	.1405	.2968	.2612	.2474	.2178	.2721	.2394
.268	.1416	.2989	.2631	.2492	.2194	.2741	.2412
.270	.1426	.3011	.2650	.2511	.2210	.2761	.2429
.272	.1436	.3032	.2669	.2529	.2226	.2781	.2447
.274	.1447	.3054	.2688	.2547	.2242	.2801	.2465
.276	.1457	.3076	.2707	.2565	.2258	.2821	.2482
.278	.1467	.3097	.2726	.2583	.2274	.2840	.2499
.280	.1478	.3119	.2745	.2602	.2290	.2861	.2517
.282	.1488	.3140	.2764	.2620	.2306	.2880	.2534
.284	.1498	.3162	.2783	.2638	.2322	.2900	.2552
.286	.1509	.3184	.2802	.2656	.2338	.2920	.2570
.288	.1519	.3205	.2821	.2674	.2354	.2940	.2587
.290	.1529	.3227	.2840	.2693	.2370	.2960	.2605
.292	.1540	.3248	.2859	.2711	.2386	.2980	.2622
.294	.1550	.3270	.2878	.2729	.2402	.3000	.2640
.296	.1560	.3292	.2897	.2747	.2418	.3020	.2658
.298	.1571	.3313	.2916	.2765	.2434	.3040	.2675
.300	.1581	.3335	.2935	.2784	.2450	.3060	.2693

43.021 *Percentages by vol. at 15.56°C (60°F) of ethyl alcohol corresponding to apparent specific gravity at various temps*[a]

Apparent specific gravity	15.56/15.56	20/20	22/22	24/24	25/25	26/26	28/28	30/30	32/32	34/34	35/35	36/36
1.0000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
.9999	.07	.07	.07	.07	.07	.07	.07	.07	.07	.07	.07	.07
98	.13	.13	.13	.13	.13	.13	.13	.13	.13	.13	.13	.13
97	.20	.20	.20	.20	.20	.20	.20	.20	.20	.20	.20	.20
96	.27	.26	.26	.26	.26	.26	.26	.26	.26	.26	.26	.26
95	.33	.33	.33	.33	.33	.33	.33	.33	.33	.33	.33	.33
94	.40	.40	.40	.40	.40	.40	.40	.40	.40	.40	.40	.40
93	.47	.46	.46	.46	.46	.46	.46	.46	.46	.46	.46	.46
92	.53	.53	.53	.53	.53	.53	.53	.53	.53	.53	.53	.53
91	.60	.60	.60	.60	.60	.60	.60	.60	.60	.60	.60	.60
90	.67	.66	.66	.66	.66	.66	.66	.66	.66	.66	66	.66
89	.73	.73	.73	.73	.73	.73	.73	.73	.73	.73	.73	.73
88	.80	.80	.80	.80	.80	.80	.79	.79	.79	.79	.79	.79
87	.87	.87	.87	.87	.87	.87	.86	.86	86	.86	.86	.86
86	.93	.93	.93	.93	.93	.93	.93	.93	.93	.93	.93	.93
85	1.00	1.00	1.00	1.00	1.00	1.00	.99	.99	.99	.99	.99	.99
84	.07	.07	.07	.07	.07	.07	1.06	1.06	1.06	1.06	1.06	1.06
83	.14	.14	.14	.13	.13	.13	.13	.13	.13	.13	.13	.13
82	.20	.20	.20	.20	.20	.20	.20	.19	.19	.19	.19	.19
81	.27	.27	.27	.27	.27	.27	.26	.26	.26	.26	.26	.26
80	.34	.34	.34	.34	.34	.33	.33	.32	.32	.32	.32	.32
79	.41	.41	.41	.40	.40	.40	.40	.39	.39	.39	.39	.39
78	.48	.48	.48	.47	.47	.47	.47	.46	.46	.46	.46	.46
77	.54	.54	.54	.54	.54	.53	.53	.53	.53	.53	.52	.52
76	.61	.61	.61	.60	.60	.60	.60	.59	.59	.59	.59	.59
75	.68	.68	.68	.67	.67	.67	.67	.66	.66	.66	.66	.66
74	.75	.75	.75	.74	.74	.73	.73	.73	.73	72	.72	.72
73	.82	.81	.81	.81	.81	.80	.80	.80	.80	.79	.79	.79
72	.88	.88	.88	.87	.87	.87	.86	.86	.86	.85	.85	.85
71	.95	.95	.95	.94	.94	.94	.93	.93	.93	.92	.92	.92
70	2.02	2.02	2.02	2.01	2.01	2.01	2.00	2.00	2.00	.99	.99	.99
69	.09	.09	.09	.08	.08	.08	.07	.07	.06	2.05	2.05	2.05
68	.16	.15	.15	.14	.14	.14	.14	.14	.13	.12	.12	.12
67	.23	.22	.22	.21	.21	.21	.20	.20	.20	.19	.19	.19
66	.30	.29	.29	.28	.28	.28	.27	.27	.27	.26	26	.26
65	.37	.36	.36	.35	.35	.35	.34	.34	.33	.32	.32	.32
64	.43	.43	.43	.42	.42	.42	.41	.41	.40	.39	.39	.39
63	.50	.50	.50	.49	.49	.49	.48	.48	.47	.46	46	.46
62	.57	.57	.57	.56	.56	.56	.55	.54	.54	.53	.53	.53
61	.64	.64	.64	.63	.63	.63	.62	.61	.60	.60	.59	.59
60	.71	.70	.70	.70	.70	.70	.69	.68	.67	.67	.66	.66
59	.78	.77	.77	.77	.77	.77	.76	.75	.74	.74	.73	.73
58	.85	.84	.84	.83	.83	.83	.82	.82	.81	.81	.80	.80
57	.92	.91	.91	.90	.90	.90	.89	.88	.87	.87	.86	.86
56	.99	.98	.98	.97	.97	.97	.96	.95	.94	.94	.93	.93
55	3.06	3.05	3.05	3.04	3.04	3.04	3.03	3.02	3.01	3.01	3.00	3.00
54	.13	.12	.12	.11	.11	.11	.10	.09	.08	.08	.07	.07
53	.20	.19	.19	.18	.18	.18	.17	.16	.15	.15	.14	.14
52	.27	.26	.26	.25	.25	.25	.24	.23	.22	.22	.21	.21
51	.34	.33	.33	.32	.32	.32	.31	.30	.29	.28	.27	.27
50	.41	.40	.40	.39	.39	.39	.38	.37	.36	.35	.34	.34
49	.49	.47	.47	.46	.46	.46	.45	.44	.43	.42	.41	.41
48	.56	.54	.54	.53	.53	.53	.52	.51	.50	.49	.48	.48
47	.63	.61	.61	.60	.60	.60	.59	.58	.57	.56	.55	.55
46	.70	.68	.68	.67	.67	.67	.66	.65	.64	.63	.62	.62
45	.77	.76	.75	.74	.74	.74	.73	.72	.70	.69	.68	.68
44	.84	.83	.82	.81	.81	.81	.79	.78	.77	.76	.75	.75
43	.91	.90	.89	.88	.88	.88	.86	.85	.84	.83	.82	.82
42	.99	.97	.96	.95	.95	.95	.93	.92	.91	.90	.89	.89
41	4.06	4.04	4.03	4.02	4.02	4.02	4.00	.99	.98	.97	.96	.96
40	.13	.11	.10	.10	.09	.09	.07	4.06	4.05	4.04	4.03	4.03
39	.20	.18	.17	.17	.16	.16	.14	.13	.12	.11	.10	.10
38	.28	.26	.25	.25	.24	.23	.21	.20	.19	.18	.17	.17
37	.35	.33	.32	.32	.31	.30	.28	.27	.26	.25	.24	.24
36	.42	.40	.39	.39	.38	.37	.36	.35	.33	.32	.31	.30
35	.50	.48	.47	.46	.45	.44	.43	.42	.40	.39	.38	.37
34	.57	.55	.54	.53	.52	.51	.50	.49	.47	.46	.45	.44
33	.64	.62	.61	.60	.59	.58	.57	.56	.54	.53	.52	.51
32	.71	.69	.68	.67	.66	.65	.64	.63	.61	.60	.59	.58
31	.79	.77	.76	.75	.74	.73	.72	.70	.68	.67	.66	.65
30	.86	.84	.83	.82	.81	.80	.79	.77	.75	.74	.73	.72

[a] Compiled at National Bureau of Standards. Table is based on data published in *Bulletin of Bureau of Standards*, Vol. 9, No. 3 (Sci. Paper No. 197).

Percentages by vol. at 15.56°C (60°F) of ethyl alcohol corresponding to apparent specific gravities at various temps—Continued. 43.021

APPARENT SPECIFIC GRAVITY	15.56/15.56	20/20	22/22	24/24	25/25	26/26	28/28	30/30	32/32	34/34	35/35	36/36
0.9930	4.86	4.84	.83	.82	4.81	.80	.79	4.77	.75	.74	4.73	.72
29	.93	.91	.90	.89	.88	.87	.86	.84	.82	.81	.80	.79
28	5.01	.98	.97	.96	.95	.94	.93	.91	.89	.88	.87	.86
27	.08	5.06	5.04	5.03	5.02	5.01	5.00	.98	.96	.95	.94	.93
26	.16	.13	.12	.11	.10	.09	.07	5.05	5.03	5.02	5.01	5.00
25	.23	.21	.19	.18	.17	.16	.14	.12	.10	.09	.08	.07
24	.31	.28	.26	.25	.24	.23	.21	.20	.18	.16	.15	14
23	.39	.36	.34	.33	.32	.31	.29	.27	.25	.23	.22	.21
22	.46	.43	.41	.40	.39	.38	.36	.34	.32	.30	.29	.28
21	.54	.51	.49	.48	.47	.46	.44	.42	.40	.38	.37	.36
20	.61	.58	.56	.55	.54	.53	.51	.49	.47	.45	.44	.43
19	.69	.66	.64	.62	.61	.60	.58	.56	.54	.52	.51	.50
18	.77	.73	.71	.70	.69	.68	.66	.64	.62	.59	.58	.57
17	.84	.81	.79	.77	76	.75	.73	.71	.69	.66	.65	.64
16	.92	.88	.86	.85	.84	.83	.80	.78	.76	.74	.73	.72
15	.99	.96	.94	.92	.91	.90	.87	.85	.83	.81	.80	.79
14	6.07	6.03	6.01	6.00	.99	.98	.95	.93	.91	.88	.87	.86
13	.15	.11	.09	.07	6.06	6.05	6.02	6.00	.98	.95	.94	.93
12	.23	.18	.16	.15	.14	.13	.10	.08	6.05	6.02	6.01	6.00
11	.30	.26	.24	.22	.21	.20	.17	.15	.12	.10	.09	.08
10	.38	.34	.32	.30	.29	.28	.25	.23	.20	.17	.16	.15
09	.46	.41	.39	.37	.36	.35	.32	.30	.28	.25	.24	.23
08	.54	.49	.47	.45	.44	.43	.40	.38	.35	.32	.31	.30
07	.62	.57	.55	.53	.52	.51	.48	.45	.42	.39	.38	.37
06	.70	.65	.63	.60	.59	.58	.55	.53	.50	.47	.46	.45
05	.77	.73	.71	.68	.67	.66	.63	.60	.57	.54	.53	.52
04	.85	.80	.78	.75	.74	.73	.70	.68	.65	.62	.60	.59
03	.93	.88	.86	.83	.82	.81	.78	.75	.72	.69	.68	.67
02	7.01	.96	.93	.90	.89	.88	.85	.83	.80	.77	.75	.74
01	.09	7.04	7.01	.98	.97	.95	.92	.90	.87	.84	.82	.81
00	.17	.12	.09	7.06	7.05	7.03	7.00	.98	.94	.91	.90	.88
0.9899	.25	.19	.16	13	.12	.10	.07	7.05	7.01	.98	.97	.95
98	.33	.27	.24	.21	.20	.18	.15	.13	.09	7.06	7.04	7.02
97	.41	.35	.32	.29	.28	.26	.23	.21	.17	.14	.12	.10
96	.50	.43	.40	.37	.36	.34	.31	.28	.24	.21	.19	.17
95	.58	.51	.48	.45	.44	.42	.39	.36	.32	.29	.27	.25
94	.66	.59	.56	.53	.52	.50	.47	.44	.40	.36	.34	.32
93	.74	.67	.64	.60	.59	.57	.54	.51	.47	.44	.42	.40
92	.82	.75	.72	.68	.67	.65	.62	.59	.55	.51	.49	.47
91	.90	.82	.79	.76	.75	.73	.70	.66	.62	.59	.57	.55
90	.98	.90	.87	.84	.83	.81	.78	.74	.70	.66	.64	.62
89	8.07	.98	.95	.92	.91	.89	.86	.82	.78	.74	.72	.70
88	.15	8.06	8.03	8.00	.98	.96	.93	.89	.85	.81	.79	.77
87	.23	.15	.11	.08	8.06	8.04	8.01	.97	.93	.89	.87	.85
86	.32	.23	.19	.16	.14	.12	.09	8.05	8.01	.96	.94	.92
85	.40	.31	.27	.24	.22	.20	.16	.12	.08	8.04	8.02	8.00
84	.48	.39	.35	.32	.30	.28	.24	.20	.16	.11	.09	.07
83	.57	.47	.43	.40	.38	.36	.32	.27	.23	.19	.17	.15
82	.65	.55	.51	.48	.46	.44	.40	.35	.31	.26	.24	.22
81	.73	.63	.59	.56	.54	.52	.48	.43	.39	.34	.32	.30
80	.82	.71	.67	.63	.61	.59	.55	.50	.46	.41	.39	.37
79	.90	.79	.75	.71	.69	.67	.63	.58	.54	.49	.47	.45
78	.98	.88	.84	.79	.77	.75	.71	.66	.61	.56	.54	.52
77	9.07	.96	.92	.87	.85	.83	.78	.73	.69	.64	.62	.60
76	.15	9.04	9.00	.95	.93	.91	.86	.81	.76	.71	.69	.67
75	.24	.13	.08	9.03	9.01	.99	.94	.89	.84	.79	.77	.75
74	.32	.21	.16	.11	.09	9.07	9.02	.96	.91	.86	.84	.82
73	.40	.29	.24	.19	.17	.15	.10	9.04	.99	.94	.92	.90
72	.49	.38	.33	.27	.25	.23	.18	.12	9.07	9.02	.99	.97
71	.57	.46	.41	.35	.33	.31	.26	.20	.15	.10	9.07	9.05
70	.66	.54	.49	.43	.41	.38	.33	.27	.22	.17	.14	.12
69	.74	.62	.57	.51	.49	.46	.41	.35	.30	.25	.22	.19
68	.82	.70	.65	.59	.57	.54	.49	.43	.37	.32	.29	.26
67	.91	.79	.74	.68	.65	.62	.57	.51	.45	.40	.37	.34
66	.99	.87	.82	.76	.73	.70	.65	.59	.53	.47	.44	.41
65	10.08	.95	.90	.84	.81	.78	.72	.66	.60	.54	.51	.48
64	.16	10.03	.98	.92	.89	.86	.80	.74	.68	.62	.59	.56
63	.25	.11	10.06	10.00	.97	.94	.88	.82	.76	.69	.66	.63
62	.33	.20	.14	.08	10.05	10.02	.96	.90	.84	.77	.74	.71
61	.42	.28	.22	.16	.13	.10	10.04	.98	.91	.84	.81	.78
60	.50	.36	.30	.24	.21	.18	.11	10.05	.99	.92	.89	.86

43.021 *Percentages by vol. at 15.56°C (60°F) of ethyl alcohol corresponding to apparent specific gravities at various temps—Continued.*

APPARENT SPECIFIC GRAVITY	15.56/15.56	20/20	22/22	24/24	25/25	26/26	28/28	30/30	32/32	34/34	35/35	36/36
0.9860	10.50	10.36	.30	24	10.21	.18	.11	10.05	.99	.92	9.89	.86
59	.59	44	.38	.32	.29	26	.19	.13	10.06	.99	.96	.93
58	68	53	.47	40	.37	.34	.27	.21	.14	10.07	10.04	10.00
57	76	61	55	48	.44	.41	.34	.28	.21	.14	.11	.07
56	.85	.69	63	56	.52	.49	.42	.36	.29	.22	.19	.15
55	.93	78	71	.64	.60	.57	.50	.44	.37	.30	.26	.23
54	11.02	.86	79	.72	.68	.65	.58	.52	.45	.38	.34	.31
53	11	94	.87	.80	.76	.73	.66	.59	.52	.45	.41	.38
52	19	11.03	.96	.88	.84	.81	.74	.67	.60	.53	.49	.45
51	28	11	11.04	.96	.92	.89	.82	.75	.67	.60	.56	.52
50	37	19	12	11.04	11.00	.96	.89	.82	.74	.67	.63	.59
49	46	28	20	.12	.08	11.04	.97	.90	.82	.75	.71	.67
48	.54	36	28	.20	.16	.12	11.05	.98	.90	.82	.78	.74
47	63	45	.36	28	.24	.20	.13	11.05	.97	.90	.86	.82
46	72	53	.45	.37	.33	.29	.21	13	11.05	.97	.93	.89
45	81	.61	.53	45	.41	.37	.29	.21	.13	11.05	11.01	.97
44	.89	70	.62	.53	.49	.45	.37	.29	.21	.12	.08	11.04
43	98	.78	.70	.61	.57	.53	.44	.36	.28	.20	.16	.12
42	12.07	.87	78	.69	.65	.61	.52	.44	.36	.27	.23	.19
41	16	.95	.86	78	.73	.69	.60	.52	.44	.35	.31	.27
40	25	12.04	.95	.86	.81	.77	.68	.60	.51	.42	.38	.34
39	.34	12	12.03	.94	.89	.85	.76	67	.58	.50	.46	.42
38	43	.21	.12	12.03	.98	.93	.84	75	.66	.57	.53	.49
37	52	.29	.20	.11	12.06	12.01	.92	.83	74	.65	.61	.57
36	61	.38	.28	.19	.14	.09	12.00	.91	.82	.73	.68	.64
35	70	47	.37	.27	.22	.17	.07	.98	.89	.80	.76	.72
34	79	.55	.45	.35	.30	.25	.15	12.06	.97	.88	.83	.79
33	88	.64	.54	.44	.39	.34	24	.14	12.05	.96	.91	.86
32	97	73	63	.52	.47	.42	32	.22	.12	12.03	.98	.93
31	13.06	.81	71	60	.55	50	.40	30	20	.11	12.06	12.01
30	16	90	.79	.68	.63	.58	48	.38	.28	.19	.14	.09
29	25	99	.88	.77	.71	.66	.56	46	.36	.26	.21	.16
28	34	13 07	96	.85	.80	.74	64	54	.44	.34	.29	.24
27	43	16	13.05	.93	.88	82	72	.62	52	.42	.37	.32
26	.52	25	.13	13.01	.96	.90	.80	.70	.59	.49	.44	.39
25	61	34	.22	.10	13.04	.99	.88	78	.67	.57	.52	.47
24	71	43	.31	.19	.13	13.08	97	.86	75	.65	.60	.55
23	80	.51	.39	.27	.21	.16	13.05	.94	.83	72	.67	.62
22	89	60	.47	.35	.29	.24	.13	13.02	.91	.80	.75	.70
21	98	68	.56	.44	.38	.33	.22	.10	.99	.88	.82	.77
20	14.08	.77	.64	.52	.46	.40	.29	.18	13.06	.95	.90	.85
19	17	.86	.73	.61	.55	.49	.37	.26	.15	13.04	.98	.93
18	26	.95	.82	.69	.63	.57	.45	.34	.22	.11	13.05	13.00
17	.36	14.04	91	.78	.72	.66	.54	.42	.30	.19	.13	.08
16	45	13	14.00	.87	.80	.74	.62	.50	.38	.27	.21	.16
15	.55	.22	.08	.95	.88	.82	.70	.58	.46	.34	.28	.23
14	64	.30	.17	14.04	.97	.91	78	.66	.54	.42	.36	.30
13	74	.39	.25	.12	14.05	.99	.86	.74	.62	.50	.44	.38
12	.83	.48	.34	.20	.13	14.07	.94	.82	.70	.58	.52	.46
11	92	.57	.43	.29	.22	.16	14.03	.90	.77	.65	.59	.53
10	15.02	.66	.51	.37	.30	.24	.11	.98	.85	.73	.67	.61
09	.11	.75	.60	.46	.39	.32	.19	14.06	.93	.81	.75	.69
08	21	.84	.69	.54	.47	.40	.27	.14	14.01	.88	.82	.76
07	.30	93	.77	.62	.55	.48	.35	.22	.09	.96	.90	.84
06	40	15.02	.86	.71	.64	.57	.43	.30	.17	14.04	.98	.92
05	.49	.11	.95	.79	.72	.65	.51	.38	.25	.12	14.05	.99
04	.58	.20	15.04	.88	.81	.74	.60	.46	.33	.20	.13	14.07
03	67	.28	.12	.96	.89	.82	.68	.54	.41	.28	.21	.15
02	77	.37	.21	15.05	.97	.90	.76	.62	.49	.36	.29	.23
01	.87	.46	.30	.14	15.06	.99	.84	.70	.56	.43	.36	.30
00	.96	.55	.39	.23	.15	15.07	.92	.78	.64	.51	.44	.38
0.9799	16.06	.64	.48	.32	.24	.16	15.01	.86	.72	.59	.52	.46
98	.15	.73	.46	.40	.32	.24	.09	.94	.80	.67	.60	.54
97	.25	.82	.55	.49	.41	.33	.17	15.02	.88	.74	.67	.61
96	.35	.91	.64	.57	.49	.41	.26	.11	.96	.82	.75	.68
95	.44	16.00	.83	.66	.58	.50	.34	.19	15.04	.90	.83	.76
94	.54	.10	.92	.75	.66	.59	.43	.27	.12	.98	.91	.84
93	.63	.19	16.01	.84	.75	.67	.51	.35	.20	15.05	.98	.91
92	.73	.28	.10	.93	.84	.76	.59	.43	.28	.13	15.06	.99
91	.83	.37	.19	16 01	.92	.84	.67	.51	.36	.21	.14	15.07
90	.92	.46	.27	.09	16.00	.92	.75	.59	.44	.29	.22	.15

Percentages by vol. at 15.56°C (60°F) of ethyl alcohol corresponding to apparent specific gravities at various temps—Continued. 43.021

Apparent Specific Gravity	15.56/15.56	20/20	22/22	24/24	25/25	26/26	28/28	30/30	32/32	34/34	35/35	36/36
0.9790	16.92	16.46	16.27	16.09	16.00	15.92	15.75	15.59	15.44	15.29	15.22	15.15
89	17.02	.55	.26	.18	.09	16.01	.84	.67	.52	.37	.30	.23
88	.12	.64	.45	.27	.18	.10	.93	.76	.61	.45	.38	.31
87	.22	.73	.54	.36	.27	.18	16.01	.84	.68	.52	.45	.38
86	.32	.83	.63	.44	.35	.26	.09	.92	.76	.60	.53	.46
85	.42	.92	.72	.53	.44	.35	.17	16.00	.84	.68	.61	.53
84	.51	17.01	.81	.62	.53	.44	.26	.08	.92	.76	.69	.61
83	.61	.10	.90	.70	.61	.52	.34	.17	.10	.84	.77	.69
82	.71	.20	.99	.79	70	.61	.43	.25	16.08	.92	.81	.76
81	81	.29	17.08	.88	78	.69	.51	.33	.16	16.00	.92	.84
80	.91	.38	.17	.97	.87	.78	.59	.41	.24	.08	16.00	.92
79	18.01	.47	.26	17.06	.96	.87	.68	.50	.33	.16	.08	16.00
78	.11	.57	.35	.14	17.04	.95	.76	.58	.41	.24	.16	.08
77	.21	.66	.44	.23	.13	17.04	.85	.66	.49	.32	.24	.16
76	.31	.75	.53	.32	.22	.12	.93	74	.57	.40	.32	.24
75	.41	.84	.62	.40	.30	.20	17.01	.83	.65	.48	.40	.32
74	.51	.94	.72	.50	.39	.29	.10	.91	.73	.56	.48	.40
73	.61	18.03	.81	.59	.48	.38	.18	.99	.81	.64	.56	.48
72	.71	.12	.90	.68	.57	.47	.27	17.07	.89	.72	.63	.55
71	.81	.22	.99	.76	.65	.55	.35	.16	.97	.80	.71	.63
70	.91	.31	18.08	.85	.74	.63	.43	.24	17.05	.88	.79	.71
69	19.01	.40	.16	.94	.83	.72	.52	.32	.14	.96	.87	.79
68	.11	.50	.25	18.02	.91	.80	.60	.40	.22	17.04	.95	.86
67	.21	.59	.34	.11	18.00	.89	.69	.49	.30	.12	17.03	.94
66	.32	.69	.44	.20	.09	.98	.78	.57	.38	.20	.11	17.02
65	.42	.78	.53	.29	.18	18.07	.86	.65	.46	.28	.19	.10
64	.52	.88	.63	.38	.27	.16	.95	.74	.55	.36	.27	.17
63	.62	.97	.71	.47	.35	.24	18.03	.82	.62	.43	.35	.25
62	.72	19.07	.81	.56	.44	.33	.11	.90	.70	.51	.43	.33
61	.83	.16	.80	.65	.53	.42	.20	.98	.78	.59	.50	.41
60	.93	.26	.99	.74	.62	.50	.28	18.07	.87	.67	.58	.49
59	20.03	.35	19.08	.83	.71	.60	.37	.15	.95	.75	.66	.56
58	.13	.45	.18	.92	.80	.69	.46	.23	18.03	.83	.74	.64
57	.23	.54	.27	19.01	.88	.77	.54	.32	.11	.91	.82	.72
56	.33	.64	.36	.10	.97	.86	.62	.40	.19	.99	.90	.80
55	.43	.73	.45	.19	19.06	.94	.70	.48	.27	18.07	.98	.88
54	.53	.83	.55	.28	.15	19.03	.79	.57	.36	.15	18.06	.96
53	.63	.92	.64	.37	.24	.12	.88	.65	.44	.23	.13	18.04
52	.73	20.02	.73	.46	.33	.21	.96	.73	.52	.31	.21	.12
51	.83	.11	.82	.55	.42	.30	19.05	.82	.60	.39	.29	.19
50	.93	.20	.91	.64	.50	.38	.13	.90	.68	.47	.37	.27
49	21.03	.30	20.01	.73	.59	.47	.22	.98	.76	.55	.45	.35
48	.13	.39	.10	.82	.68	.56	.31	19.07	.85	.64	.53	.43
47	.23	.48	.19	.91	.77	.65	.39	.15	.93	.72	.61	.51
46	.33	.58	.28	20.00	.86	.74	.48	.24	19.01	.80	.69	.59
45	.43	.67	.37	.09	.95	.82	.56	.32	.09	.88	.77	.67
44	52	.76	.46	.17	20.03	.90	.64	.40	.17	.96	.85	.75
43	.62	.86	.55	.26	.12	.99	.73	.49	.26	19.04	.93	.83
42	.72	.95	.64	.35	.21	20.08	.82	.57	.34	.12	19.01	.91
41	.82	21.04	.73	.44	.30	.17	.91	.66	.42	.20	.09	.98
40	.92	.14	.82	.53	.38	.25	.99	.74	.50	.28	.17	19.06
39	22.02	.23	.91	.62	.47	.34	20.07	.82	.58	.35	.24	.23
38	.12	.32	21.00	.71	.56	.43	.16	.90	.66	.43	.32	.31
37	.22	.41	.09	.79	.64	.51	.24	.98	.74	.51	.40	.29
36	.31	.50	.18	.88	.73	.59	.32	20.06	.82	.59	.48	.37
35	.41	.60	.27	.97	.82	.68	.41	.15	.90	.67	.56	.45
34	.51	.69	.36	21.05	90	.77	.50	:24	.99	.75	.64	.53
33	.61	78	.45	.14	.99	.85	.58	.32	20.07	.83	72	61
32	.71	.87	.54	.23	21.08	.94	.66	.40	.15	.91	.80	.68
31	.80	.96	.63	.32	.16	21.02	.74	.48	.23	.99	.87	.76
30	.90	22.05	.72	.41	.25	11	.83	.56	.31	20.07	.95	.84
29	23.00	.14	.81	.50	.34	.20	.91	.64	.39	.15	20.03	.92
28	.10	.24	.90	.58	.42	.28	.99	.72	.47	.23	.11	20.00
27	.19	.33	.99	.67	.51	.36	21.07	.80	.55	.31	.19	.08
26	.29	.42	22.08	.76	.59	.45	.16	.89	.63	.39	.27	.16
25	.38	.51	.17	.84	.68	.53	.24	.97	.71	.46	.34	.23
24	.48	.60	.26	.93	.77	.62	.33	21.05	.79	.54	.42	.30
23	.58	.69	.34	22.01	.85	.70	.41	.13	.87	.62	.50	.38
22	.67	.78	.43	.10	.94	.78	.49	.21	.95	.70	.58	.46
21	.77	.87	.52	.19	22.03	.87	.58	.30	21.03	.78	.66	.54
20	.87	.96	.61	.27	.11	.96	.66	.38	.11	.86	.73	.61

43.021 *Percentages by vol. at 15.56°C (60°F) of ethyl alcohol corresponding to apparent specific gravities at various temps*—Continued.

APPARENT SPECIFIC GRAVITY	15.56/15.56	20/20	22/22	24/24	25/25	26/26	28/28	30/30	32/32	34/34	35/35	36/36
0.9720	23.87	22.96	.61	.27	22.11	.96	.66	21.38	11	.86	20.73	.61
19	.96	23.06	.70	.36	.19	22.04	.74	.46	.19	.94	.81	.69
18	24.06	.15	.79	.45	.28	.12	.82	.54	.27	21.02	.89	.77
17	.15	.24	.88	.54	.36	.21	.91	.62	.35	.10	.97	.85
16	.25	.33	.96	.62	.45	.30	.99	.70	.43	17	21.05	.92
15	.34	.42	23.05	.70	.53	.38	22.08	.79	.51	24	.12	.99
14	.43	.51	.14	.79	.62	.46	.16	.87	.59	33	.20	21.08
13	.53	.60	.22	.87	.70	.54	.24	.95	.67	40	27	.15
12	.62	.69	.31	.96	.79	.63	.32	22.03	.75	.88	.35	.22
11	.72	.78	.40	23.04	.87	.71	.40	.11	.83	.56	.43	.30
10	.81	.87	.49	.13	.96	.80	.49	.19	.91	.64	.50	.37
09	.91	.95	.57	.21	23.04	.88	.57	.27	.99	.72	.58	.45
08	25.00	24.04	.66	.30	.13	.97	.65	.35	22.07	.80	.66	.53
07	.09	.13	.74	.38	.21	23.05	.73	.43	.14	.87	.73	.60
06	.19	.22	.83	.47	.29	.13	.81	.51	.22	.95	.81	.68
05	.28	.31	.92	.56	.38	.22	.90	.59	30	22.03	.89	.76
04	.38	.40	24.00	.64	.46	.30	.98	.67	.38	.10	.96	.83
03	.47	.49	.09	.73	.55	.38	23.06	.75	.46	18	22.04	.91
02	.57	.58	.18	.81	.63	.46	.14	.83	.53	.25	.11	.98
01	.66	.66	.26	.89	.71	.54	.21	.90	.61	.33	.19	22.06
00	.75	.75	.35	.98	.80	.63	.30	.98	.69	41	.27	.14
0.9699	.85	.84	.44	24.06	.88	.72	.38	23.06	.77	.48	.34	.21
98	.94	.93	.53	.15	.97	.80	.46	.14	.84	.55	.42	.28
97	26.04	25.01	.61	.23	24.05	.88	.54	.22	.92	.63	.49	.35
96	.13	.10	.69	.31	.13	.96	.62	.30	23.00	.71	.57	.43
95	.22	.19	.78	.40	.22	24.05	.70	.38	.08	.78	.64	.50
94	.31	.28	.86	.48	.30	.13	.78	.45	.15	.86	.72	.58
93	.41	.36	.95	.57	.38	.21	.86	.53	.23	.94	.80	.66
92	.50	.45	25.04	.65	.47	.29	.94	.61	.31	23.01	.87	.74
91	.59	.54	.13	.74	.55	.37	24.02	.69	.38	.08	.95	.81
90	.69	.62	.21	.82	.63	.45	.10	.77	.46	.16	23.02	.88
89	.78	.71	.29	.90	.72	.53	.18	.84	.53	.23	.10	.96
88	.87	.80	.38	.98	.80	.61	.26	.92	.61	.31	.17	23.03
87	.96	.89	.46	25.07	.88	.69	.34	24.00	.68	.38	.24	.10
86	27.05	.98	.55	.15	.97	.77	.42	.08	.76	.46	.32	.18
85	.15	26.06	.63	.23	25.05	.85	.50	.16	.84	.53	.39	.25
84	.24	.15	.72	.32	.13	.94	.58	.23	.92	.61	.47	.33
83	.33	.24	.80	.40	.21	25.02	.66	.31	.99	.68	.54	.40
82	.42	.33	.89	.48	.29	.10	.74	.39	24.06	75	.61	.47
81	.51	.41	.97	.57	.37	.18	.81	.47	.14	.83	.69	.54
80	.60	.50	26.06	.65	.45	.26	.89	.54	.21	.90	.76	.61
79	.69	.59	.14	.73	.53	.34	.97	.62	.30	.98	.84	.69
78	.78	.67	.22	.81	.61	.42	25.05	.70	.37	24.06	.91	.77
77	.87	.76	.31	.89	.69	.50	.13	.78	.45	.14	.99	.84
76	.96	.84	.39	.97	.77	.58	.21	.85	.52	.21	24.06	.91
75	28.05	.93	.47	26.05	.85	.66	.29	.93	.60	.29	.13	.99
74	.14	27.01	.56	.14	.94	.74	.37	25.01	.68	.36	.21	24.06
73	.23	.10	.64	.22	26.02	.82	.45	.09	.75	.43	.28	.13
72	.32	.19	.73	.30	.10	.90	.53	.16	.83	.51	.36	.20
71	.41	.27	.81	.38	.18	.98	.60	.24	.90	.58	.43	.28
70	.50	.36	.89	.46	.26	26.06	.68	.32	.98	.66	.50	.35
69	.59	.44	.97	.54	.34	.14	.76	.40	25.06	.73	.58	.42
68	.68	.52	27.05	.63	.42	.22	.84	.47	.13	.81	.65	.50
67	.77	.61	.14	.71	.50	.30	.92	.55	.20	.88	.73	.57
66	.86	.69	.22	.79	.58	.38	.99	.63	.28	.95	.80	.64
65	.95	.77	.30	.87	.66	.46	26.07	.70	.36	25.03	.87	.72
64	29.04	.86	.39	.95	.74	.54	.15	.78	.44	.11	.95	.79
63	.12	.94	.47	27.03	.82	.62	.23	.86	.51	.18	25.02	.86
62	.21	28.02	.55	.11	.90	.70	.31	.94	.59	.25	.09	.93
61	.30	.11	.64	.19	.98	.77	.38	26.02	.66	.33	.17	25.01
60	.39	.19	.72	.27	27.06	.85	.46	.09	.74	.40	.24	.08
59	.47	.28	.81	.35	.13	.93	.54	.17	.82	.48	.31	.15
58	.56	.36	.89	.43	.21	27.01	.61	.24	.89	.56	.39	.23
57	.65	.44	.97	.51	.29	.09	.69	.32	.97	.63	.46	.30
56	.74	.53	27.05	.59	.37	.17	.77	.39	26.04	.70	.53	.37
55	.82	.61	.13	.67	.45	.25	.85	.47	.11	.77	.61	.45
54	.91	.69	.21	.75	.53	.33	.93	.55	.19	.85	.68	.52
53	30.00	.78	.29	.83	.61	.41	27.00	.62	.26	.92	.75	.59
52	.09	.86	.37	.91	.69	.49	.08	.70	.34	.99	.82	.66
51	.17	.94	.45	.99	.77	.56	.16	.78	.41	26.06	.90	.74
50	.26	29.03	.53	28.07	.85	.64	.23	.85	.49	.14	.97	.81

Percentages by vol. at 15.56°C (60°F) of ethyl alcohol corresponding to apparent specific gravities at various temps—Continued. **43.021**

APPARENT SPECIFIC GRAVITY	15.56/15.56	20/20	22/22	24/24	25/25	26/26	28/28	30/30	32/32	34/34	35/35	36/36
0.9650	30.26	29.03	.53	.07	27.85	.64	.23	26.85	.49	.14	25.97	.81
49	.34	.11	.61	.15	.93	.72	.31	.92	.56	.21	26.04	.89
48	.43	.19	.69	.23	28.01	.79	.38	27.00	.64	.29	.11	.96
47	.52	.27	.73	.31	.09	.87	.46	.07	.71	.36	.19	26.03
46	.60	.35	.85	.39	.16	.95	.53	.15	.78	.43	.26	.10
45	.69	.44	.93	.47	.24	28.03	.61	.22	.85	.51	.3	.17
44	.78	.52	29.02	.55	.32	.10	.69	.30	.93	.58	.40	.24
43	.86	.60	.10	.63	.40	.18	.76	.37	27.00	.65	.47	.31
42	.95	.68	.18	.71	.47	.26	.84	.44	.07	.72	.54	.38
41	31.03	.76	.26	.79	.55	.34	.91	.52	.14	.79	.61	.45
40	.11	.85	.34	.86	.63	.41	.99	.59	.22	.86	.69	.52
39	.20	.93	.42	.93	.71	.49	28.06	.67	.29	.93	.76	.59
38	.28	30.01	.50	29.10	.78	.56	.14	.74	.37	27.01	.83	.66
37	.36	.09	.58	.09	.86	.64	.21	.81	.44	.08	.90	.73
36	.44	.17	.66	.17	.94	.72	.29	.89	.51	.15	.97	.80
35	.52	.25	.74	.25	29.02	.80	.37	.96	.58	.22	27.04	.87
34	.61	.34	.28	.33	.09	.87	.44	28.04	.66	.29	.11	.94
33	.69	.42	.90	.41	.17	.95	.52	.11	.73	.36	.18	27.01
32	.77	.50	.98	.49	.25	29.03	.60	.19	.80	.43	.25	.08
31	.85	.58	30.06	.57	.33	.11	.67	.26	.87	.50	.32	.15
30	.93	.66	.13	.64	.40	.18	.74	.33	.95	.58	.39	.22
29	32.02	.74	.21	.72	.48	.26	.82	.41	28.02	.65	.46	.29
28	.09	.82	.29	.79	.56	.33	.89	.48	.0 1	.72	.54	.36
27	.17	.89	.36	.87	.64	.41	.97	.56	.17	.79	.61	.43
26	.25	.97	.44	.95	.71	.48	29.04	.63	.24	.86	.68	.50
25	.33	31.05	.52	30.03	.79	.56	.12	.70	.31	.93	.75	.57
24	.41	.13	.60	.10	.87	.64	.20	.78	.38	28.00	.82	.64
23	.49	.20	.67	.17	.95	.71	.27	.85	.45	.07	.89	.71
22	.57	.28	.75	.25	30.02	.79	.35	.93	.52	.14	.96	.78
21	.65	.36	.83	.33	.10	.86	.42	29.00	.59	.21	28.03	.85
20	.72	.44	.91	.41	.17	.94	.50	.07	.67	.29	.10	.92
19	.80	.52			.25	30.01	.57	.14	.74	.36	.17	.99
18	.88	.59			.32	.09	.65	.22	.82	.43	.24	28.06
17	.96	.67			.40	.16	.72	.29	.89	.50	.31	.13
16	33.04	.75			.47	.24	.79	.36	.96	.57	.3 8	.20
15	.12	.82			.54	.31	.86	.43	29.03	.64	.45	.27
14	.19	.90			.62	.39	.94	.51	.10	.71	.52	.43
13	.27	.98			.69	.46	30.01	.58	.17	.78	.59	.41
12	.35	32.05			.77	.53	.08	.65	.24	.85	.66	.48
11	.43	.13			.84	.61	.15	.72	.31	.92	.73	.55
10	.50	.21			.92	.68	.23	.80	.39	.99	.80	.62
09	.58	.28			.99	.75	.30	.87	.46	29.06	.87	.69
08	.66	.36			31.07	.83	.38	.49	.53	.13	.94	.76
07	.74	.43			.13	.90	.45	30.01	.60	.20	29.01	.83
06	.81	.51			.21	.98	.52	.09	.67	.27	.08	.90
05	.89	.58			.92	31.05	.59	.16	.74	.34	.15	.97
04	.97	.66			.36	.13	.66	.23	.81	.41	.22	29.04
03	34.05	.73			.43	.20	.73	.30	.88	.48	.29	.11
02	.12	.81			.51	.28	.80	.37	.95	.55	.36	.18
01	.20	.88			.58	.35	.88	.44	30.02	.62	.43	.25
00	.27	.96			.65	.42	.95	.51	.09	.69	.50	.31
0.9599	.35	33.03			.73			.58	.16	.76	.57	.38
98	.42	.10			.80			.65	.23	.83	.63	.45
97	.50	.18			.87			.72	.30	.90	.70	.51
96	.57	.25			.95			079	.37	.97	.77	.58
95	.65	.32			32.02			.87	.44	30.04	.84	.65
94	.72	.40			.09			.94	.51	.11	.91	72
93	.80	.47			.16			31.01	.58	.18	.98	.79
92	.87	.54			.23			.08	.65	.25	30.05	.86
91	.95	.62			.30			.15	.72	.32	.12	.93
90	35.02	.69			.37			.22	.79	.38	.18	.99
89	.09	.76			.44			.38			.25	30.06
88	.17	.84			.51			.35			.23	.13
87	.24	.91			.58			.42			.39	.20
86	.31	.98			.65			.49			.46	.27
85	.38	34.05			.73			.56			.52	.33
84	.46	.12			.80			.63			.59	.40
83	.53	.20			.87			.70			.66	.47
82	.60	.27			.94			.77			.73	.54
81	.67	.34			33.01			.84			.80	.61
80	.75	.41			.08			.91			.86	.67

43.021 *Percentages by vol. at 15.56°C (60°F) of ethyl alcohol corresponding to apparent specific gravities at various temps*—Continued.

APPARENT SPECIFIC GRAVITY	15.56/15.56	20/20	25/25	30/30	35/35	APPARENT SPECIFIC GRAVITY	15.56/15.56	20/20	25/25	30/30	35/35
0.9580	35.75	34.41	33.08	31.91	30.86	0.9510	40.46	39.10	37.71	36.47	35.34
79	.82	.48	.15	.98	.93	09	.52	.16	.78	.53	.40
78	.89	.56	.22	32.05	31.00	08	.58	.23	.84	.59	.46
77	.96	.63	.29	.11	.07	07	.65	.29	.90	.65	.52
76	36.04	.70	.36	.18	13	06	.71	.35	.96	.72	.58
75	.11	.77	.43	.25	.20	05	.77	.41	38.02	.78	.64
74	.18	.84	.50	.32	.26	04	.84	.48	.09	.84	.71
73	.25	.91	.57	.38	.33	03	.90	.54	.15	.90	.77
72	.32	.98	.64	.45	.39	02	.96	.60	.21	.96	.83
71	.39	35.05	.71	.52	46	01	41.02	.67	.27	37.02	.89
70	.46	.12	.78	.58	53	00	.09	.73	.33	.09	.95
69	.53	.19	.85	.65	59	0.9499	.15	.79	.40	.15	36.01
68	.60	.26	.92	.72	66	98	.21	.85	.46	.21	.07
67	.67	.33	.99	.79	72	97	.27	.91	.52	.27	.13
66	.74	.40	34.05	.85	79	96	.33	.98	.58	.33	.19
65	.81	.47	12	.92	.86	95	.40	40.04	.64	.39	.25
64	.88	.54	19	.99	.92	94	.46	.10	.70	.45	.31
63	.95	.61	.26	33.05	99	93	.52	.16	.77	.51	.37
62	37.02	.68	.32	.12	32.05	92	.58	.22	.83	.57	.43
61	.09	.75	.39	19	12	91	.64	.29	.89	.63	.49
60	16	.82	.46	25	18	90	.70	.35	.95	.70	.55
59	.22	.88	.53	.32	.25	89	.77	.41	39.01	.76	.61
58	.29	.95	.59	.39	.31	88	.83	.47	.07	.82	.67
57	.36	36.02	.66	.45	.37	87	.89	.53	.13	.88	.73
56	.43	.09	.73	.52	.44	86	.95	.59	.20	.94	.79
55	.50	.15	.80	.59	.50	85	42.01	.65	.26	38.00	.85
54	.56	.22	.86	.65	.57	84	.07	.71	.32	.06	.91
53	.63	.29	.93	.72	.63	83	.13	.78	.38	.12	.97
52	.70	.36	35.00	.79	.70	82	.19	.84	.44	.18	37.03
51	.77	.42	.07	.85	.76	81	.25	.90	.50	.24	.09
50	.84	.49	.13	.92	.83	80	.31	.96	.56	.30	.15
49	.90	.56	.20	.99	.89	79	.37	41.02	.62	.36	.21
48	.97	.63	.26	34.05	.95	78	.43	.08	.68	.42	.26
47	38.04	.69	.33	.12	33.02	77	.49	.14	.74	.48	.32
46	.11	.76	.39	.18	.08	76	.55	.20	.80	.54	.38
45	.17	.83	.46	.25	.15	75	.61	.26	.87	.60	.44
44	.24	.89	.53	.31	.21	74	.67	.32	.93	.66	.50
43	.31	.96	.59	.38	.27	73	.73	.38	.99	.72	.56
42	.37	37.03	.66	.44	.34	72	.80	.44	40.05	.78	.62
41	.44	.09	.72	.51	.40	71	.86	.50	.11	.84	.68
40	.51	.16	.79	.57	.46	70	.92	.56	.17	.90	.74
39	.57	.23	.86	.64	.53	69	.98	.62	.22	.96	.79
38	.64	.29	.92	.70	.59	68	43.04	.68	.28	39.02	.85
37	.71	.36	.99	.77	.66	67	.09	.74	.34	.08	.91
36	.77	.42	36.05	.83	.72	66	.15	.80	.40	.13	.97
35	.84	.49	.12	.90	.78	65	.21	.86	.46	.19	38.03
34	.91	.56	.18	.96	.85	64	.27	.92	.52	.25	.09
33	.97	.62	.25	35.03	.91	63	.33	.98	.58	.31	.15
32	39.04	.69	.31	.09	.97	62	.39	42.04	.64	.37	.20
31	.10	.75	.38	.15	34.04	61	.45	.09	.70	.43	.26
30	.17	.82	.44	.22	.10	60	.51	.15	.76	.49	.32
29	.23	.88	.51	.28	.16	59	.57	.21	.82	.54	.38
28	.30	.95	.57	.34	.22	58	.63	.27	.88	.60	.44
27	.36	38.01	.64	.41	.29	57	.69	.33	.93	.66	.49
26	.43	.07	.70	.47	.35	56	.75	.39	.99	.72	.55
25	.49	.14	.77	.53	.41	55	.80	.45	41.05	.78	.61
24	.56	.20	.83	.59	.47	54	.86	.51	.11	.84	.67
23	.62	.27	.90	.66	.53	53	.92	.57	.17	.89	.73
22	.69	.33	.96	.72	.60	52	.98	.63	.23	.95	.78
21	.75	.39	37.02	.78	.66	51	44.04	.69	.28	40.01	.84
20	.82	.46	.09	.85	.72	50	.10	.74	.34	.07	.90
19	.88	.52	.15	.91	.78	49	.16	.80	.40	.13	.96
18	.95	.59	.21	.97	.84	48	.21	.86	.46	.18	39.02
17	40.01	.65	.28	36.04	.91	47	.27	.92	.51	.24	.07
16	.08	.72	.34	.10	.97	46	.33	.98	.57	.30	.13
15	.14	.78	.40	.16	35.04	45	.39	43.04	.63	.35	.19
14	.20	.84	.46	.22	.10	44	.45	.09	.69	.41	.24
13	.27	.91	.52	.28	.16	43	.50	.15	.75	.47	.30
12	.33	.97	.59	.35	.22	42	.56	.21	.80	.53	.36
11	.39	39.04	.65	.41	.28	41	.62	.27	.86	.58	.41
10	.46	.10	.71	.47	.34	40	.68	.33	.92	.64	.47

Percentages by vol. at 15.56°C (60°F) of ethyl alcohol corresponding to apparent specific gravities at various temps—Continued. **43.021**

APPARENT SPECIFIC GRAVITY	15.56 / 15.56	20/20	25/25	30/30	35/35	APPARENT SPECIFIC GRAVITY	15.56 / 15.56	20/20	25/25	30/30	35/35
0.9440	44.68	43.33	41.92	40.64	39.47	0.9370	48.53	47.20	45.81	44.52	43.33
39	.73	.39	.98	.70	.53	69	.58	.26	.86	.58	.38
38	.79	.44	42.03	.75	.59	68	.63	.31	.91	.63	.43
37	.85	.50	.09	.81	.64	67	.69	.36	.97	.68	.49
36	.91	.56	.15	.87	.70	66	.74	.42	46.02	.74	.54
35	.97	.62	.21	.93	.76	65	.79	.47	.07	.79	.59
34	45.02	.67	.26	.98	.81	64	.85	.52	.13	.84	.65
33	.08	.73	.32	41.04	.87	63	.90	.58	.18	.90	.70
32	.14	.78	.38	.10	.93	62	.95	.63	.23	.95	.75
31	.19	.85	.43	.15	.98	61	49.01	.68	.29	45.01	.81
30	.25	.90	.49	.21	40.04	60	.06	.73	.34	.06	.86
29	.31	.96	.55	.27	.09	59	.11	.79	.39	.11	.91
28	.36	44.02	.61	.32	.15	58	.16	.84	.45	.16	.97
27	.42	.07	.66	.38	.21	57	.21	.89	.50	.22	44.02
26	.47	.13	.72	.44	.26	56	.26	.94	.55	.27	.07
25	.53	.18	.78	.49	.32	55	.32	48.00	.61	.32	.13
24	.59	.24	.83	.55	.37	54	.37	.05	.66	.37	.18
23	.64	.30	.89	.60	.43	53	.42	.10	.71	.43	.23
22	.70	.35	.95	.66	.48	52	.47	.15	.77	.48	.28
21	.76	.41	43.01	.72	.54	51	.52	.21	.82	.53	.34
20	.81	.46	.06	.77	.59	50	.58	.26	.87	.58	.39
19	.87	.52	.12	.83	.65	49	.63	.31	.93	.64	.44
18	.93	.58	.17	.89	.71	48	.68	.36	.98	.69	.49
17	.98	.63	.23	.94	.76	47	.73	.41	47.03	.74	.54
16	46.04	.69	.29	42.00	.82	46	.78	.47	.08	.79	.60
15	.09	.74	.34	.06	.87	45	.83	.52	.14	.85	.65
14	.15	.80	.40	.11	.93	44	.89	.57	.19	.90	.70
13	.20	.86	.46	.17	.98	43	.94	.62	.24	.95	.75
12	.26	.91	.51	.22	41.04	42	.99	.68	.29	46.01	.81
11	.31	.97	.57	.28	.09	41	50.04	.73	.34	.06	.86
10	.37	45.03	.62	.33	.15	40	.09	.78	.40	.11	.91
09	.43	.08	.68	.39	.20	39	.14	.83	.45	.16	.96
08	.48	.14	.74	.44	.26	38	.19	.88	.50	.21	45.02
07	.54	.19	.79	.50	.31	37	.24	.94	.55	.27	.07
06	59	.25	.85	.56	.37	36	.30	.99	.60	.32	.12
05	65	.30	.90	.61	.42	35	.35	49.04	.66	.37	.17
04	.70	.36	.96	.67	.48	34	.40	.09	.71	.42	.22
03	.76	.42	44.02	.72	.53	33	.45	.14	.76	.47	.27
02	.81	.47	.07	.78	.59	32	.50	.19	.81	.53	.33
01	.87	.53	.13	.83	.64	31	.55	.25	.86	.58	.38
00	.92	.58	.18	.89	.70	30	.60	.30	.92	.63	.43
0.9399	.98	64	.23	.94	.75	29	.65	.35	.97	.68	.48
98	47 03	69	.29	43.00	.81	28	.70	.40	48.02	.73	.53
97	.09	.74	.34	.05	.86	27	.75	.45	.07	.79	.59
96	.14	.80	.40	.11	.92	26	.81	.50	.12	.84	.64
95	.19	.85	.45	.16	.97	25	.86	.55	.17	.89	.69
94	.25	.91	.51	.22	42.03	24	.91	.60	.22	.94	.74
93	.30	.96	.56	.27	.08	23	.96	.65	.28	.99	.79
92	.35	46.01	.62	.33	.14	22	51.01	.70	.33	47.05	.84
91	.41	.07	.67	.38	.19	21	.06	.75	.38	.10	.90
90	.46	.12	.73	.44	.24	20	.11	.80	.43	.15	.95
89	.52	.18	.78	.49	.30	19	.16	.85	.48	.20	46.00
88	.57	.23	.84	.55	.35	18	.21	.90	.53	.25	.05
87	.62	.29	.89	.60	.41	17	.26	.95	.58	.30	.10
86	.68	.34	.95	.66	.46	16	.31	50.00	.63	.35	.15
85	.73	.39	45.00	.71	.52	15	.36	.05	.68	.40	.20
84	.78	.45	.05	.77	.57	14	.41	.10	.73	.45	.26
83	.84	.50	.11	.82	.63	13	.46	.16	.79	.50	.31
82	.89	.56	.16	.87	.68	12	.51	.21	.84	.55	.36
81	.95	.61	.22	.93	.73	11	.56	.26	.89	.60	.41
80	48.00	.67	.27	.98	.79	10	.61	.31	.94	.65	.46
79	.05	.72	.32	44.04	.84	09	.66	.36	.99	.71	.51
78	.11	.77	.38	.09	.90	08	.71	.41	49.04	.76	.56
77	.16	.83	.43	.15	.95	07	.76	.46	.09	.81	.61
76	.21	.88	.48	.20	43.01	06	.81	.51	.14	.86	.66
75	.26	.94	.54	.25	.06	05	.86	.56	.19	.91	.71
74	.32	.99	.59	.31	.11	04	.91	.61	.24	.96	.77
73	.37	47.04	.65	.36	.17	03	.96	.66	.29	48.01	.82
72	.42	.10	.70	.41	.22	02	52.01	.71	.34	.06	.87
71	.48	.15	.75	.47	.27	01	.06	.76	.39	.11	.92
70	.53	.20	.81	.52	.33	00	.11	.81	.44	.16	.97

43.021 *Percentages by vol. at 15.56°C (60°F) of ethyl alcohol corresponding to apparent specific gravities at various temps*—Continued.

APPARENT SPECIFIC GRAVITY	15.56/15.56	20/20	25/25	30/30	35/35	APPARENT SPECIFIC GRAVITY	15.56/15.56	20/20	25/25	30/30	35/35
0.9300	52.11	50.81	49.44	48.16	46.97	0.9230	55.52	54.24	52.88	51.61	50.41
0.9299	.16	.86	.49	.21	47.02	29	.57	.29	.93	.66	.46
98	.21	.91	.54	.26	.07	28	.62	.33	.98	.71	.51
97	.26	.96	.59	.31	.12	27	.67	.38	53.03	.75	.56
96	.31	51.01	.64	.36	.17	26	.71	.43	.08	.80	.60
95	.36	.06	.69	.41	.22	25	.76	.48	.12	.85	.65
94	.41	11	.74	.46	.27	24	.81	.53	.17	.90	.70
93	.46	.16	.79	.51	.32	23	.86	.57	.22	.95	.75
92	.51	.21	.84	.56	.37	22	.90	.62	.27	52.00	.80
91	.56	.26	.89	.61	.42	21	.95	.67	.31	.04	.85
90	.61	.31	.94	.66	.47	20	56.00	.72	.36	.09	.89
89	.66	.36	.99	.71	.52	19	.05	.77	.41	.14	.94
88	.71	.41	50.04	.76	.57	18	.09	.81	.46	.19	.99
87	.76	.46	.09	.81	.62	17	.14	.86	.50	.23	51.04
86	.81	.50	.14	.86	.67	16	.19	.91	.55	.28	.09
85	.86	.55	.19	.91	.72	15	.24	.96	.60	.33	.13
84	.91	.60	.24	.96	.77	14	.28	55.00	.65	.38	.18
83	.96	.65	.29	49.01	.82	13	.33	.05	.70	.43	.23
82	53.00	.70	.34	.06	.87	12	.38	.10	.74	.47	.27
81	.05	.75	.39	.11	.92	11	.43	.15	.79	.52	.32
80	.10	.80	.44	.16	.97	10	.47	.19	.84	.57	.37
79	.15	.85	.49	.21	48.02	09	.52	.24	.89	.62	.42
78	.20	.90	.54	.26	.07	08	.57	.29	.93	.67	.46
77	.25	.95	.59	.31	.12	07	.62	.34	.98	.71	.51
76	.30	52.00	.64	.36	.17	06	.66	.38	54.03	.76	.56
75	.35	.05	.68	.41	.22	05	.71	.43	.08	.81	.61
74	.40	.10	.73	.46	.27	04	.76	.48	.12	.86	.65
73	.45	.15	.78	.51	.32	03	.81	.53	.17	.90	.70
72	.50	.20	.83	.56	.37	02	.85	.57	.22	.95	.75
71	.54	.25	.88	.61	.42	01	.90	.62	.26	53.00	.80
70	.59	.29	.93	.66	.47	00	.95	.67	.31	.05	.84
69	.64	.34	.98	.71	.52	0.9199	57.00	.71	.36	.09	.89
68	.69	.39	51.03	.76	.57	98	.04	.76	.41	.14	.94
67	.74	.44	.08	.81	.62	97	.09	.81	.45	.19	.99
66	.79	.49	.13	.86	.67	96	.13	.86	.50	.23	52.03
65	.84	.54	.18	.91	.71	95	.18	.90	.55	.28	.08
64	.89	.59	.23	.96	.76	94	.23	.95	.59	.33	.13
63	.94	.64	.27	50.00	.81	93	.27	56.00	.64	.37	.17
62	.99	.69	.32	.05	.86	92	.32	.04	.69	.42	.22
61	54.03	.74	.37	.10	.91	91	.37	.09	.74	.47	.27
60	.08	.79	.42	.15	.96	90	.41	.14	.78	.51	.32
59	.13	.84	.47	.20	49.01	89	.46	.18	.83	.56	.36
58	.18	.89	.52	.25	.06	88	.51	.23	.88	.61	.41
57	.23	.93	.57	.30	.11	87	.55	.28	.92	.65	.46
56	.28	.98	.62	.35	.15	86	.60	.32	.97	.70	.50
55	.32	53.03	.67	.40	.20	85	.65	.37	55.02	.75	.55
54	.37	.08	.72	.44	.25	84	.69	.42	.07	.79	.60
53	.42	.13	.76	.49	.30	83	.74	.46	.11	.84	.65
52	.47	.18	.81	.54	.35	82	.79	.51	.16	.89	.69
51	.52	.22	.86	.59	.40	81	.83	.56	.21	.93	.74
50	.57	.27	.91	.64	.44	80	.88	.60	.25	.98	.79
49	.61	.32	.96	.69	.49	79	.93	.65	.30	54.03	.83
48	.66	.37	52.01	.74	.54	78	.97	.70	.35	.07	.88
47	.71	.42	.06	.79	.59	77	58.02	.74	.39	.12	.93
46	.76	.47	.11	.83	.64	76	.06	.79	.44	.17	.98
45	.81	.52	.16	.88	.69	75	.11	.84	.49	.21	53.02
44	.86	.56	.20	.93	.73	74	.16	.88	.53	.26	.07
43	.90	.61	.25	.98	.78	73	.20	.93	.58	.31	.12
42	.95	.66	.30	51.03	.83	72	.25	.97	.63	.36	.16
41	55.00	.71	.35	.08	.88	71	.29	57.02	.67	.40	.21
40	.05	.76	.40	.13	.93	70	.34	.07	.72	.45	.26
39	.10	.81	.45	.17	.98	69	.38	.11	.77	.50	.30
38	.14	.85	.50	.22	50.02	68	.43	.16	.81	.54	.35
37	.19	.90	.54	.27	.07	67	.47	.21	.86	.59	.40
36	.24	.95	.59	.32	.12	66	.52	.25	.91	.64	.44
35	.29	54.00	.64	.37	.17	65	.57	.30	.95	.68	.49
34	.33	.05	.69	.42	.22	64	.61	.35	56.00	.73	.53
33	.38	.09	.74	.46	.27	63	.66	.39	.05	.78	.58
32	.43	.14	.79	.51	.31	62	.70	.44	.09	.82	.63
31	.48	.19	.83	.56	.36	61	.75	.48	.14	.87	.67
30	.52	.24	.88	.61	.41	60	.79	.53	.18	.92	.72

43.021 *Percentages by vol. at 15.56°C (60°F) of ethyl alcohol corresponding to apparent specific gravities at various temps*—Continued.

Apparent specific gravity	15.56/15.56	20/20	25/25	30/30	35/35	Apparent specific gravity	15.56/15.56	20/20	25/25	30/30	35/35
0.9160	58.79	57.53	56.18	54.92	53.72	0.9090	61.92	60.68	59.36	58.11	56.93
59	.84	.58	.23	.96	.77	89	.96	.72	.40	.15	.97
58	.89	.62	.28	55.01	.81	88	62.01	.77	.45	.20	57.02
57	.93	.67	.32	.06	.86	87	.05	.81	.49	.24	.06
56	.98	.71	.37	.10	.91	86	.10	.86	.54	.29	.11
55	59.02	.76	.41	.15	.95	85	.14	.90	.58	.33	.15
54	.07	.81	.46	.19	54.00	84	.18	.94	.63	.38	.19
53	.11	.85	.51	.24	.05	83	.23	.99	.67	.42	.24
52	.16	.90	.55	.29	.09	82	.27	61.03	.71	.46	.28
51	.20	.94	.60	.33	.14	81	.31	.08	.76	.51	.33
50	.25	.99	.65	.38	.18	80	.36	.12	.80	.55	.37
49	.29	58.03	.69	.42	.23	79	.40	.17	.85	.60	.42
48	.34	.08	.74	.47	.28	78	.45	.21	.89	.64	.46
47	.38	.13	.78	.52	.32	77	.49	.25	.94	.69	.50
46	.43	.17	.83	.56	.37	76	.53	.30	.98	.73	.55
45	.47	.22	.88	.61	.41	75	.58	.34	60.03	.77	.59
44	.52	.26	.92	.65	.46	74	.62	.39	.07	.82	.64
43	.56	.31	.97	.70	.51	73	.66	.43	.11	.86	.68
42	.61	.35	57.01	.75	.55	72	.71	.47	.16	.91	.73
41	.65	.40	.06	.79	.60	71	.75	.52	.20	.95	.77
40	.70	.44	.10	.84	.65	70	.79	.56	.25	59.00	.81
39	.74	.49	.15	.88	.69	69	.84	.60	.29	.04	.86
38	79	.53	.20	.93	.74	68	.88	.65	.33	.08	.90
37	.83	.58	.24	.98	.78	67	.93	.69	.38	.13	.95
36	.88	.62	.29	56.02	.83	66	.97	.74	.42	.17	.99
35	.92	.67	.33	.07	.88	65	63.01	.78	.46	.21	58.04
34	.97	.71	.38	.11	.92	64	.06	.82	.51	.26	.08
33	60.01	.76	.42	.16	.97	63	.10	.87	.55	.30	.12
32	.06	.80	.47	.21	55.01	62	.14	.91	.60	.35	.17
31	.10	.85	.51	.25	.06	61	.19	.96	.64	.39	.21
30	.15	.89	.56	.30	.11	60	.23	62.00	.68	.43	.26
29	.19	.94	.60	.34	.15	59	.27	.04	.73	.48	.30
28	.24	.98	.65	.39	.20	58	.32	.09	.77	.52	.34
27	.28	59.03	.70	44	.24	57	.36	.13	.82	.57	.39
26	.33	.07	.74	.48	.29	56	.40	.17	.86	.61	.43
25	.37	.12	.79	.53	.33	55	.45	.22	.90	.65	.48
24	.42	.16	.83	.57	.38	54	.49	.26	.95	.70	.52
23	.46	.21	.88	.62	.42	53	.53	.30	.99	.74	.56
22	.50	.25	.92	.67	.47	52	.58	.35	61.03	.79	.61
21	.55	.30	.97	.71	.52	51	.62	.39	.08	.83	.65
20	.59	.34	58.01	.76	.56	50	.66	.43	.12	.87	.70
19	.64	.39	.06	.80	.61	49	.71	.48	.16	.92	.74
18	.68	.43	.10	.85	.65	48	.75	.52	.21	.96	.78
17	.73	.48	.15	.89	.70	47	.79	.56	.25	60.00	.83
16	.77	.52	.19	.94	.74	46	.84	.60	.29	.05	.87
15	.82	.57	.24	.99	.79	45	.88	.65	.34	.09	.92
14	.86	.61	.28	57 03	.84	44	.92	.69	.38	.14	.96
13	.91	.66	.33	.08	.88	43	.97	.73	.42	.18	59.00
12	.95	.70	.37	12	.93	42	64.01	.78	.47	.22	.05
11	61.00	.75	.42	.17	.97	41	.05	.82	.51	.27	.09
10	.04	.79	.46	.21	56.02	40	.09	.86	.55	.31	.13
09	.08	.84	.51	.26	.06	39	.14	.91	.60	.35	.18
08	.13	.88	.55	.30	.11	38	.18	.95	.64	.40	.22
07	.17	.92	.60	.35	.15	37	.22	.99	.68	.44	.27
06	.22	.97	.64	.39	.20	36	.27	63.04	.73	.48	.31
05	.26	60.01	.69	.44	.25	35	.31	.08	.77	.53	.35
04	.30	.06	.73	.48	.29	34	.35	.12	.81	.57	.40
03	.35	.10	.78	.53	.34	33	.40	.17	.86	.62	.44
02	.39	.15	.82	.57	.38	32	.44	.21	.90	.66	.48
01	.44	.19	.87	.62	.43	31	.48	.25	.94	.70	.53
00	.48	.24	.91	.66	.47	30	.53	.30	.99	.75	.57
0.9099	.52	.28	.96	.71	.52	29	.57	.34	62.03	.79	.61
98	.57	.33	59.00	.75	.56	28	.61	.38	.07	.83	.66
97	.61	.37	.04	.80	.61	27	.66	.43	.12	.88	.70
96	.66	.41	.09	.84	.65	26	.70	.47	.16	.92	.74
95	.70	.46	.13	.89	.70	25	.74	.51	.20	.97	.79
94	.74	.50	.18	.93	.75	24	.78	.56	.24	61.01	.83
93	.79	.55	.22	.98	.79	23	.83	.60	.29	.05	.87
92	.83	.59	.27	58.02	.84	22	.87	.64	.23	.09	.92
91	.88	.64	.31	.07	.88	21	.91	.69	.37	.14	.96
90	.92	.68	.36	.11	.93	20	.96	.73	.42	.18	60.00

43.021 *Percentages by vol. at 15.56°C (60°F) of ethyl alcohol corresponding to apparent specific gravities at various temps*—Continued.

APPARENT SPECIFIC GRAVITY	15.56/15.56	20/20	25/25	30/30	35/35
0.9020	64.96	63.73	62.42	61.18	60.00
19	65.00	.77	.46	.22	.05
18	.04	.82	.50	.27	.09
17	.09	.86	.55	.31	.13
16	.13	.90	.59	.35	.18
15	.17	.94	.63	.40	.22
14	.21	.99	.67	.44	.26
13	.26	64.03	.72	.48	.30
12	.30	.07	.76	.53	.35
11	.34	.11	.80	.57	.39
10	.38	.16	.85	.61	.43
09	.43	.20	89	.66	.48
08	.47	.24	.93	.70	.52
07	.51	.28	.97	.74	.56
06	.55	.33	63.02	.78	.61
05	.60	.37	.06	.83	.65
04	.64	.41	.10	.87	.69
03	.68	.45	.15	.91	.73
02	.72	.50	.19	.96	.78
01	.77	.54	.23	62.00	.82
00	.81	.58	.27	.04	.86
0.8999	.85	.62	.32	.09	.91
98	.89	.67	.36	.13	.95
97	.94	.71	.40	.17	.99
96	.98	.75	.44	.21	61.03
95	66.02	.79	.49	.26	.08
94	.06	.84	.53	.30	.12
93	.10	.88	.57	.34	.16
92	.15	.92	.62	.38	.21
91	.19	.96	.66	.43	.25
90	.23	65.01	.70	.47	.29
89	.27	.05	.74	.51	.33
88	.31	.09	.79	.55	.38
87	.36	.13	.83	.60	42
86	.40	.18	.87	.64	.46
85	.44	.22	.91	.68	.50
84	.48	.26	.96	.72	.55
83	.52	.30	64.00	77	.59
82	.56	.35	.04	.81	.63
81	.61	.39	.08	.85	.68
80	.65	.43	.13	.89	.72
79	.69	.47	.17	.94	.76
78	.73	.51	.21	.98	.80
77	.77	.56	.25	63.02	.85
76	.82	.60	.30	.06	.89
75	.86	.64	.34	.11	.93
74	.90	.68	.38	.15	.97
73	.94	.72	.42	.19	62.02
72	.98	.77	.47	.23	.06
71	67.03	.81	.51	.28	.10
70	.07	.85	55	.32	.14
69	.11	.89	.59	.36	.19
68	.15	.94	.64	.40	.23
67	.19	.98	.68	.44	.27
66	.23	66.02	.72	.49	.31
65	.28	.06	.76	.53	.36
64	.32	.10	.81	.57	.40
63	.36	.15	.85	.61	.44
62	.40	.19	.89	.66	.48
61	.44	.23	.93	.70	.53
60	.48	.27	.97	.74	.57
59	.53	.31	65.02	.78	.61
58	.57	.36	.06	.83	.65
57	.61	.40	.10	.87	.69
56	.65	.44	.14	.91	.74
55	.69	.48	.18	.95	.78
54	.73	.52	.23	64.00	.82
53	.78	.56	.27	.04	.86
52	.82	.60	.31	.08	.91
51	.86	.65	.35	.12	.95
50	.90	.69	.39	.16	.99

APPARENT SPECIFIC GRAVITY	15.56/15.56	20/20	25/25	30/30	35/35
0.8950	67.90	66.69	65.39	64.16	62.99
49	.94	.73	.44	.21	63.03
48	.98	.77	.48	.25	.08
47	68.02	.81	.52	.29	.12
46	.07	.85	.56	.33	.16
45	.11	.90	.60	.37	.20
44	.15	.94	.64	.42	.24
43	.19	.98	.69	.46	.28
42	.23	67.02	.73	.50	.33
41	.27	.06	.77	.54	.37
40	.31	.10	.81	.58	.41
39	.35	.15	.85	.63	.45
38	.39	.19	.90	.67	.49
37	.43	.23	.94	.71	.54
36	.48	.27	.98	.75	.58
35	.52	.31	66.02	.79	.62
34	.56	.35	.06	.84	.66
33	.60	.39	.10	.88	.70
32	.64	.43	.15	.92	.74
31	.68	.47	.19	.96	.79
30	.72	.52	.23	65.00	.83
29	.76	.56	.27	.05	.87
28	.80	.60	.31	.09	.91
27	.84	.64	.35	.13	.95
26	.89	.68	.39	.17	64.00
25	.93	.72	.44	.21	.04
24	.97	.76	.48	.25	.08
23	69.01	.80	.52	.29	.12
22	.05	.84	.56	.34	.16
21	.09	.89	.60	.38	.21
20	.13	.93	.64	.42	.25
19	.17	.97	.68	.46	.29
18	.21	68.01	.73	.50	.33
17	.25	.05	.77	.54	.37
16	.29	.09	.81	.59	.41
15	.33	.13	.85	.63	.46
14	.37	.17	.89	.67	.50
13	.41	.21	.93	.71	.54
12	.46	.26	.98	.75	.58
11	.50	.30	67.02	.79	.62
10	.54	.34	.06	.83	.67
09	.58	.38	.10	.88	.71
08	.62	.42	.14	.92	.75
07	.66	.46	.18	.96	.79
06	.70	.50	.22	66.00	.83
05	.74	.54	.26	.04	.87
04	.78	.58	.30	.08	.92
03	.82	.62	.34	.12	.96
02	.86	.67	.39	.17	65.00
01	90	.71	.43	.21	.04
00	.94	.75	.47	.25	.08
0.8899	.98	.79	.51	.29	.12
98	70.02	.83	.55	.33	.17
97	.06	.87	.59	.37	.21
96	10	.91	.63	.41	.25
95	.14	.95	.67	.45	.29
94	18	.99	.71	.50	.33
93	.22	69.03	.75	.54	.37
92	.27	.07	.80	.58	.41
91	.31	.11	.84	.62	.45
90	.35	.15	.88	.66	.50
89	.39	.19	.92	.70	.54
88	.43	.23	96	.74	.58
87	.47	.27	68.00	.79	.62
86	.51	.32	.04	.83	.66
85	.55	.36	.08	.87	.70
84	.59	.40	.12	.91	.74
83	.63	.44	.16	.95	.79
82	.67	.48	.20	.99	.83
81	.71	.52	.24	67.03	.87
80	.75	.56	.28	.07	.91

Percentages by vol. at 15.56°C (60°F) of ethyl alcohol corresponding to apparent specific gravities at various temps—Continued. 43.021

APPARENT SPECIFIC GRAVITY	15.56/15.56	20/20	25/25	30/30	35/35	APPARENT SPECIFIC GRAVITY	15.56/15.56	20/20	25/25	30/30	35/35
0.8880	70.75	69.56	68.28	67.07	65.91	0.8810	73.50	72.34	71.09	69.89	68.74
79	.79	.60	.33	.11	.95	09	.54	.38	.13	.93	.78
78	.83	.64	.37	.15	.99	08	.58	.42	.16	.97	.82
77	.87	.68	.41	.20	66.03	07	.62	.46	.20	70.01	.86
76	.91	.72	.45	.24	.07	06	.66	.50	.24	.05	.90
75	.95	.76	.49	.28	.11	05	.70	.53	.28	.09	.94
74	.99	.80	.53	.32	.16	04	.74	.57	.32	.13	.98
73	71.03	.84	.57	.36	.20	03	.78	.61	.36	.17	69.02
72	.07	.88	.61	.40	.24	02	.81	.65	.40	.21	.06
71	.11	.92	.65	.44	.28	01	.85	.69	.44	.25	.10
70	.15	.96	.69	.48	.32	00	.89	.73	.48	.29	.14
69	.19	70.00	.73	.52	.36	0.8799	.93	.77	.52	.33	.18
68	.23	.04	.77	.56	.40	98	.97	.81	.56	.37	.22
67	.27	.08	.81	.60	.44	97	74.01	.85	.60	.41	.26
66	.31	.12	.85	.64	.48	96	.05	.88	.64	.44	.30
65	.35	.16	.89	.68	.52	95	.08	.92	.67	.48	.34
64	.38	.20	.93	.72	.56	94	.12	.96	.71	.52	.38
63	.42	.24	.98	.76	.60	93	.16	73.00	.75	.56	.42
62	.46	.28	69.02	.80	.64	92	.20	.04	.79	.60	.45
61	.50	.32	.06	.85	.69	91	.24	.08	.83	.64	.49
60	.54	.36	.10	.89	.73	90	.28	.12	.87	.68	.53
59	.58	.40	.14	.93	.77	89	.32	.16	.91	.72	.57
58	.62	.44	.18	.97	.81	88	.36	.19	.95	.76	.61
57	.66	.48	.22	68.01	.85	87	.39	.23	.99	.80	.65
56	.70	.52	.26	.05	.89	86	.43	.27	72.03	.84	.69
55	.74	.56	.30	.09	.93	85	.47	.31	.07	.88	.73
54	.78	.60	.34	.13	.97	84	.51	.35	.11	.92	.77
53	.82	.64	.38	.17	67.01	83	.55	.39	.14	.96	.81
52	.86	.68	.42	.21	.05	82	.59	.43	.18	71.00	.85
51	.90	.72	.46	.25	.09	81	.63	.47	.22	.04	.89
50	.94	.76	.50	.29	.13	80	.66	.50	.26	.07	.93
49	.98	.80	.54	.33	.17	79	.70	.54	.30	.11	.97
48	72.02	.84	.58	.37	.21	78	.74	.58	.34	.15	70.01
47	.06	.88	.62	.41	.25	77	.78	.62	.38	.19	.05
46	.10	.92	.66	.45	.29	76	.82	.66	.42	.23	.09
45	.14	.96	.70	.49	.33	75	.86	.70	.46	.27	.13
44	.18	71.00	.74	.53	.38	74	.90	.74	.49	.31	.16
43	.22	.04	.78	.57	.42	73	.93	.78	.53	.35	.20
42	.25	.08	.82	.61	.46	72	.97	.81	.57	.39	.24
41	.29	.12	.86	.65	.50	71	75.01	.85	.61	.42	.28
40	.33	.16	.90	.69	.54	70	.05	.89	.65	.46	.32
39	.37	.20	.94	.73	.58	69	.09	.93	.69	.50	.36
38	.41	.24	.98	.77	.62	68	.13	.97	.73	.54	.40
37	.45	.27	70.02	.81	.66	67	.16	74.01	.77	.58	.44
36	.49	.31	.06	.85	.70	66	.20	.05	.81	.62	.48
35	.53	.35	.10	.89	.74	65	.24	.08	.84	.66	.52
34	.57	.39	.13	.93	.78	64	.28	.12	.88	.70	.56
33	.61	.43	.17	.97	.82	63	.32	.16	.92	.74	.60
32	.65	.47	.21	69.01	.86	62	.35	.20	.96	.77	.64
31	.69	.51	.25	.05	.90	61	.39	.24	73.00	.81	.67
30	.73	.55	.29	.09	.94	60	.43	.28	.04	.85	.71
29	.76	.59	.33	.13	.98	59	.47	.32	.08	.89	.75
28	.80	.63	.37	.17	68.02	58	.51	.35	.12	.93	.79
27	.84	.67	.41	.21	.06	57	.54	.39	.15	.97	.83
26	.88	.71	.45	.25	.10	56	.58	.43	.19	72.01	.87
25	.92	.75	.49	.29	.14	55	.62	.47	.23	.05	.91
24	.96	.79	.53	.33	.18	54	.66	.51	.27	.08	.95
23	73.00	.83	.57	.37	.22	53	.70	.55	.31	.12	.99
22	.04	.87	.61	.41	.26	52	.73	.58	.35	.16	71.03
21	.08	.91	.65	.45	.30	51	.77	.62	.38	.20	.07
20	.12	.95	.69	.49	.34	50	.81	.66	.42	.24	.10
19	.16	.99	.73	.53	.38	49	.85	.70	.46	.28	.14
18	.19	72.03	.77	.57	.42	48	.89	.74	.50	.32	.18
17	.23	.07	.81	.61	.46	47	.92	.77	.54	.36	.22
16	.27	.10	.85	.65	.50	46	.96	.81	.58	.39	.26
15	.31	.14	.89	.69	.54	45	76.00	.85	.62	.43	.30
14	.35	.18	.93	.73	.58	44	.04	.89	.65	.47	.34
13	.39	.22	.97	.77	.62	43	.07	.93	.69	.51	.38
12	.43	.26	71.01	.81	.66	42	.11	.97	.73	.55	.41
11	.47	.30	.05	.85	.70	41	.15	75.00	.77	.59	.45
10	.50	.34	.09	.89	.74	40	.19	.04	.81	.63	.49

43.021 *Percentages by vol. at 15.56°C (60°F) of ethyl alcohol corresponding to apparent specific gravities at various temps*—Continued.

APPARENT SPECIFIC GRAVITY	15.56/15.56	20/20	25/25	30/30	35/35
0.8740	76.19	75.04	73.81	72.63	71.49
39	.22	.08	.85	.66	.53
38	.26	.12	.88	.70	.57
37	.30	.16	.92	.74	.61
36	.34	.19	.96	.78	.65
35	.37	.23	74.00	.82	.69
34	.41	.27	.04	.86	.72
33	.45	.31	.08	.90	.76
32	.49	.35	.11	.93	.80
31	.52	.38	.15	.97	.84
30	.56	.42	.19	73.01	.88
29	.60	.46	.23	.05	.92
28	.64	.50	.27	.09	.96
27	.67	.54	.31	.13	72.00
26	.71	.57	.34	.16	.03
25	.75	.61	.38	.20	.07
24	.79	.65	.42	.24	.11
23	.82	.69	.46	.28	.15
22	.86	.73	.50	.32	.19
21	.90	.76	.53	.35	.23
20	.94	.80	.57	.39	.27
19	.97	.84	.61	.43	.30
18	77.01	.88	.65	.47	.34
17	.05	.91	.69	.51	.38
16	.09	.95	.73	.55	.42
15	.12	.99	.76	.58	.46
14	.16	76.03	.80	.62	.50
13	.20	.06	.84	.66	.53
12	.23	.10	.88	.70	.57
11	.27	.14	.92	.74	.61
10	.31	.18	.95	.77	.65
09	.34	.22	.99	.81	.69
08	.38	.25	75.03	.85	.73
07	.42	.29	.07	.89	.77
06	.46	.33	.10	.93	.80
05	.49	.37	.14	.97	.84
04	.53	.40	.18	74.00	.88
03	.57	.44	.22	.04	.92
02	.60	.48	.25	.08	.96
01	.64	.52	.29	.12	73.00
00	.68	.55	.33	.16	.03
0.8699	.71	.59	.37	.19	.07
98	.75	.63	.40	.23	.11
97	.79	.66	.44	.27	.15
96	.83	.70	.48	.31	.19
95	.86	.74	.52	.35	.22
94	.90	.78	.55	.38	.26
93	.94	.81	.59	.42	.30
92	.97	.85	.63	.46	.34
91	78.01	.89	.67	.50	.38
90	.05	.92	.70	.54	.41
89	.08	.96	.74	.57	.45
88	.12	77.00	.78	.61	.49
87	.16	.03	.82	.65	.53
86	.19	.07	.85	.69	.56
85	.23	.11	.89	.73	.60
84	.27	.14	.93	.76	.64
83	.30	.18	.97	.80	.68
82	.34	.22	76.00	.84	.72
81	.38	.26	.04	.88	.75
80	.41	.29	.08	.92	.79
79	.45	.33	.12	.95	.83
78	.49	.37	.15	.99	.87
77	.52	.40	.19	75.03	.91
76	.56	.44	.23	.07	.94
75	.60	.48	.26	.10	.98
74	.63	.51	.30	.14	74.02
73	.67	.55	.34	.18	.06
72	.71	.59	.38	.22	.09
71	.74	.62	.41	.25	.13
70	.78	.66	.45	.29	.17

APPARENT SPECIFIC GRAVITY	15.56/15.56	20/20	25/25	30/30	35/35
0.8670	78.78	77.66	76.45	75.29	74.17
69	.82	.70	.49	.33	.21
68	.85	.73	.53	.37	.24
67	.89	.77	.56	.40	.28
66	.93	.81	.60	.44	.32
65	.96	.84	.64	.48	.36
64	79.00	.88	.68	.51	.39
63	.04	.92	.71	.55	.43
62	.07	.96	.75	.59	.47
61	.11	.99	.79	.63	.51
60	.14	78.03	.82	.66	.55
59	.18	.07	.86	.70	.58
58	.22	.10	.90	.74	.62
57	.25	.14	.94	.78	.66
56	.29	.17	.97	.81	.70
55	.32	.21	77.01	.85	.73
54	.36	.25	.05	.89	.77
53	.40	.28	.08	.93	.81
52	.43	.32	.12	.96	.85
51	.47	.36	.16	76.00	.88
50	.51	.39	.19	.04	.92
49	.54	.43	.23	.07	.96
48	.58	.47	.27	.11	75.00
47	.61	.50	.30	.15	.03
46	.65	.54	.34	.19	.07
45	.69	.57	.38	.22	.11
44	.72	.61	.41	.26	.15
43	.76	.65	.45	.30	.18
42	.79	.68	.49	.33	.22
41	.83	.72	.52	.37	.26
40	.87	.76	.56	.41	.29
39	.90	.79	.60	.44	.33
38	.94	.83	.63	.48	.37
37	.97	.86	.67	.52	.41
36	80.01	.90	.71	.56	.44
35	.05	.94	.74	.59	.48
34	.08	.97	.78	.63	.52
33	.12	79.01	.82	.67	.56
32	.15	.05	.85	.70	.59
31	.19	.08	.89	.74	.63
30	.22	.12	.93	.78	.67
29	.26	.16	.96	.81	.71
28	.30	.19	78.00	.85	.74
27	.33	.23	.04	.89	.78
26	.37	.26	.07	.93	.82
25	.40	.30	.11	.96	.85
24	.44	.34	.14	77.00	.89
23	.47	.37	.18	.04	.93
22	.51	.41	.22	.07	.97
21	.55	.45	.25	.11	76.00
20	.58	.48	.29	.15	.04
19	.62	.52	.33	.18	.08
18	.65	.55	.36	.22	.11
17	.69	.59	.40	.26	.15
16	.72	.63	.43	.29	.19
15	.76	.66	.47	.33	.23
14	.80	.70	.51	.36	.26
13	.83	.73	.54	.40	.30
12	.87	.77	.58	.44	.34
11	.90	.80	.62	.47	.37
10	.94	.84	.65	.51	.41
09	.97	.88	.69	.55	.45
08	81.01	.91	.72	.58	.48
07	.05	.95	.76	.62	.52
06	.08	.98	.80	.66	.56
05	.12	80.02	.83	.69	.59
04	.15	.05	.87	.73	.63
03	.19	.09	.91	.77	.67
02	.22	.13	.94	.80	.70
01	.26	.16	.98	.84	.74
00	.29	.20	79.01	.88	.78

Percentages by vol. at 15.56°C (60°F) of ethyl alcohol corresponding to apparent specific gravities at various temps—Continued. **43.021**

Apparent specific gravity	15.56/15.56	20/20	25/25	30/30	35/35	Apparent specific gravity	15.56/15.56	20/20	25/25	30/30	35/35
0.8600	81.29	80.20	79.01	77.88	76.78	0.8530	83.73	82.66	81.50	80.38	79.30
0.8599	.33	.23	.05	.91	.82	29	.77	.69	.54	.42	.34
98	.36	.27	.09	.95	.85	28	.80	.73	.57	.45	.38
97	.40	.30	.12	.99	.89	27	.84	.76	.61	.49	.41
96	.43	.34	.16	78.02	.93	26	.87	.80	.64	.52	.45
95	.47	.38	19	.06	.96	25	.90	.83	.68	.56	.48
94	.51	.41	.23	.09	77.00	24	.94	.87	.71	.59	.52
93	.54	.45	.27	.13	.04	23	.97	.90	.75	.63	.55
92	.58	.48	.30	.17	.07	22	84.01	.94	.78	.66	.59
91	.61	.52	.34	.20	.11	21	.04	.97	.82	.70	.62
90	.65	.55	37	.24	.14	20	.07	83.01	.85	.73	.66
89	.68	.59	.41	.27	.18	19	.11	.04	.89	.77	.70
88	.72	.62	.44	.31	.22	18	.14	.07	.92	.81	.73
87	.75	.66	48	.35	.25	17	.18	.11	.96	.84	.77
86	.79	.69	.52	.38	.29	16	.21	.14	.99	.88	.80
85	.82	.73	.55	.42	.33	15	.24	.18	82.03	.91	.84
84	.86	.77	.59	.45	.36	14	.28	.21	.06	95	.87
83	.89	.80	.62	.49	.40	13	.31	.25	.10	.98	.91
82	.93	.84	.66	.53	.43	12	.34	.28	.13	81.02	.94
81	.96	.87	.70	.56	.47	11	.38	.32	.16	.05	.98
80	82.00	.91	.73	.60	.51	10	.41	.35	.20	.09	80.01
79	.03	.94	.77	.64	.54	09	.45	.39	.23	.12	.05
78	.07	.98	.80	.67	.58	08	.48	.42	.27	.16	.08
77	.10	81.01	.84	.71	.62	07	.51	.45	.30	.19	.12
76	.14	.05	.87	.74	.65	06	.55	.49	.34	.23	.15
75	.17	.08	.91	.78	.69	05	.58	.52	.37	.26	.19
74	.21	.12	.95	.82	.72	04	.61	.56	.41	.30	.23
73	.24	.16	.98	.85	.76	03	.65	.59	.44	.33	.26
72	.28	.19	80.02	.89	.80	02	.68	.62	.47	.37	.30
71	.31	.23	.05	.92	.83	01	.72	.66	.51	.40	.33
70	.35	.26	.09	.96	.87	00	.75	.69	.54	.44	.37
69	.38	.30	.12	79.00	.91	0.8499	.78	.73	.58	.47	.40
68	.42	.33	.16	.03	.94	98	.82	.76	.61	.51	.44
67	.45	.37	.20	.07	.98	97	.85	.79	.65	.54	.47
66	.49	.40	.23	.10	78.01	96	.89	.83	.68	.57	.51
65	.52	.44	.27	.14	.05	95	.92	.86	.71	.61	.54
64	.56	.47	.30	.17	.09	94	.95	.90	.75	.64	.58
63	.59	.51	.34	.21	.12	93	.99	.93	.78	.68	.61
62	.63	.54	.37	.25	.16	92	85.02	.97	.82	.71	.65
61	.66	.58	.41	.28	.19	91	.05	84.00	.85	.75	.68
60	.70	.61	.44	.32	.23	90	.09	.03	.89	.78	.72
59	.73	.65	.48	.35	.27	89	.12	.07	.92	.82	.75
58	.77	.68	.51	.39	.30	88	.15	.10	.96	.85	.79
57	.80	.72	.55	.42	.34	87	.18	.14	.99	.89	.82
56	.84	.75	.59	.46	.37	86	.22	.17	83.02	.92	.86
55	.87	.79	.62	.49	.41	85	.25	.20	.06	.96	.89
54	.91	.82	.66	.53	.45	84	.28	.24	.09	.99	.93
53	.94	.86	.69	.57	.48	83	.32	.27	.13	82.03	.96
52	.98	.89	.73	.60	.52	82	.35	.31	.16	.06	81.00
51	83.01	.93	.76	.64	.55	81	.38	.34	.20	.10	.03
50	.04	.96	.80	.67	.59	80	.42	.37	.23	.13	.07
49	.08	82.00	.83	.71	.63	79	.45	.41	.26	.17	.10
48	.11	.03	.87	.74	.66	78	.48	.44	.30	.20	.14
47	.15	.07	.90	.78	.70	77	.51	.47	.33	.24	.17
46	.18	.10	.94	.81	.73	76	.55	.51	.37	.27	.21
45	.22	.14	.98	.85	.77	75	.58	.54	.40	.30	.24
44	.25	.17	81.01	.89	.81	74	.61	.57	.43	.34	.28
43	.29	.21	.05	.92	.84	73	.65	.61	.47	.37	.31
42	.32	.24	.08	.96	.88	72	.68	.64	.50	.41	.35
41	.35	.28	.12	.99	.91	71	.71	.67	.54	.44	.38
40	.39	.31	.15	80.03	.95	70	.75	.71	.57	.48	.42
39	.42	.35	.19	.06	.99	69	.78	.74	.61	.51	.45
38	.46	.38	.22	.10	79.02	68	.81	.78	.64	.55	.49
37	.49	.42	.26	.13	.06	67	.84	.81	.67	.58	.52
36	.53	.45	.29	.17	.09	66	.88	.84	.71	.62	.56
35	.56	.49	.30	.20	.13	65	.91	.88	.74	.65	.59
34	.59	.52	.36	.24	.16	64	.94	.91	.78	.69	.63
33	.63	.55	.40	.28	.20	63	.98	.94	.81	.72	.66
32	.66	.59	.43	.31	.23	62	86.01	.98	.85	.75	.70
31	.70	.62	.47	.35	.27	61	.04	85.01	.88	.79	.73
30	.73	.66	.50	.38	.30	60	.08	.04	.91	.82	.77

43.021 *Percentages by vol. at 15.56°C (60°F) of ethyl alcohol corresponding to apparent specific gravities at various temps*—Continued.

APPARENT SPECIFIC GRAVITY	15.56/15.56	20/20	25/25	30/30	35/35	APPARENT SPECIFIC GRAVITY	15.56/15.56	20/20	25/25	30/30	35/35
0.8460	86.08	85.04	83.91	82.82	81.77	0.8390	88.33	87.33	86.24	85.18	84.16
59	.11	.08	.95	.86	.80	89	.36	.36	.28	.22	.19
58	.14	.11	.98	.89	.84	88	.39	.39	.31	.25	.22
57	.17	.14	84.02	.93	.87	87	.43	.43	.34	.28	.26
56	.21	.18	.05	.96	.91	86	.46	.46	.37	.31	.29
55	.24	.21	.08	83.00	.94	85	.49	.49	.40	.35	.32
54	.27	.24	.12	.03	.98	84	.52	.52	.44	.38	.36
53	.30	.28	.15	.06	82.01	83	.55	.55	.47	.41	.39
52	.34	.31	.18	.10	.04	82	.58	.58	.50	.45	.42
51	.37	.34	.22	.13	.08	81	.61	.62	.53	.48	.46
50	.40	.38	.25	.17	.11	80	.65	.65	.57	.51	.49
49	.43	.41	.29	.20	.15	79	.68	.68	.60	.54	.52
48	.47	.44	.32	.23	.18	78	.71	.71	.63	.58	.55
47	.50	.48	.35	.27	.22	77	.74	.74	.66	.61	.59
46	.53	.51	.39	.30	.25	76	.77	.78	.70	.64	.62
45	.57	.54	.42	.34	.28	75	.80	.81	.73	.68	.65
44	.60	.57	.45	.37	.32	74	.83	.84	.76	.71	.69
43	.63	.61	.49	.40	.35	73	.87	.87	.79	.74	.72
42	.66	.64	.52	.44	.39	72	.90	.90	.83	.77	.75
41	.70	.67	.55	.47	.42	71	.93	.94	.86	.81	.79
40	.73	.71	.59	.51	.46	70	.96	.97	.89	.84	.82
39	.76	.74	.62	.54	.49	69	.99	88.00	.92	.87	.85
38	.79	.77	.65	.57	.52	68	89.02	.03	.95	.90	.89
37	.83	.80	.69	.61	.56	67	.05	.06	.99	.94	.92
36	.86	.84	.72	.64	.59	66	.08	.09	87.02	.97	.95
35	.89	.87	.76	.68	.63	65	.11	.13	.05	86.00	.99
34	.92	.90	.79	.71	.66	64	.14	.16	.08	.04	85.02
33	.96	.94	.82	.74	.70	63	.18	.19	.11	.07	.05
32	.99	.97	.86	.78	.73	62	.21	.22	.15	.10	.08
31	87.02	86.00	.89	.81	.76	61	.24	.25	.18	.13	.12
30	.05	.03	.92	.85	.80	60	.27	.29	.21	.16	.15
29	.09	.07	.96	.88	.83	59	.30	.32	.24	.20	.18
28	.12	.10	.99	.91	.87	58	.33	.35	.27	.23	.22
27	.15	.13	85.02	.95	.90	57	.36	.38	.31	.26	.25
26	.18	.16	.06	.98	.93	56	.39	.41	.34	.29	.28
25	.22	.20	.09	84.02	.97	55	.42	.44	.37	.33	.31
24	.25	.23	.12	.05	83.00	54	.45	.47	.40	.36	.35
23	.28	.26	.16	.08	.04	53	.48	.50	.43	.39	.38
22	.31	.30	.19	.12	.07	52	.51	.54	.46	.42	.41
21	.34	.33	.22	.15	.11	51	.54	.57	.50	.45	.44
20	.38	.36	.25	.18	.14	50	.58	.60	.53	.49	.48
19	.41	.39	.29	.22	.17	49	.61	.63	.56	.52	.51
18	.44	.43	.32	.25	.21	48	.64	.66	.59	.55	.54
17	.47	.46	.35	.28	.24	47	.67	.69	.62	.58	.58
16	.50	.49	.39	.32	.28	46	.70	.72	.66	.62	.61
15	.54	.52	.42	.35	.31	45	.73	.75	.69	.65	.64
14	.57	.56	.45	.38	.34	44	.76	.79	.72	.68	.67
13	.60	.59	.49	.42	.38	43	.79	.82	.75	.71	.71
12	.63	.62	.52	.45	.41	42	.82	.85	.78	.75	.74
11	.67	.65	.55	.48	.45	41	.85	.88	.82	.78	.77
10	.70	.68	.59	.52	.48	40	.88	.91	.85	.81	.80
09	.73	.72	.62	.55	.51	39	.91	.94	.88	.84	.84
08	.76	.75	.65	.59	.55	38	.94	.97	.91	.87	.87
07	.79	.78	.69	.62	.58	37	.98	89.00	.94	.91	.90
06	.83	.81	.72	.65	.62	36	90.01	.04	.97	.94	.93
05	.86	.85	.75	.69	.65	35	.04	.07	88.01	.97	.97
04	.89	.88	.78	.72	.68	34	.07	.10	.04	87.00	86.00
03	.92	.91	.82	.75	.72	33	.10	.13	.07	.04	.03
02	.95	.94	.85	.79	.75	32	.13	.16	.10	.07	.06
01	.99	.98	.88	.82	.79	31	.16	.19	.13	.10	.10
00	88.02	87.01	.92	.85	.82	30	.19	.22	.16	.13	.13
0.8399	.05	.04	.95	.89	.85	29	.22	.25	.19	.16	.16
98	.08	.07	.98	.92	.89	28	.25	.28	.23	.19	.19
97	.11	.10	86.02	.95	.92	27	.28	.31	.26	.23	.23
96	.14	.14	.05	.99	.96	26	.31	.35	.29	.26	.26
95	.18	.17	.08	85.02	.99	25	.34	.38	.32	.29	.29
94	.21	.20	.11	.05	84.02	24	.37	.41	.35	.32	.32
93	.24	.23	.15	.09	.06	23	.40	.44	.38	.35	.35
92	.27	.27	.18	.12	.09	22	.43	.47	.41	.39	.39
91	.30	.30	.21	.15	.12	21	.46	.50	.45	.42	.42
90	.33	.33	.24	.18	.16	20	.49	.53	.48	.45	.45

Percentages by vol. at 15.56°C (60°F) of ethyl alcohol corresponding to apparent specific gravities at various temps—Continued. **43.021**

Apparent specific gravity	15.56/15.56	20/20	25/25	30/30	35/35	Apparent specific gravity	15.56/15.56	20/20	25/25	30/30	35/35
0.8320	90.49	89.53	88.48	87.45	86.45	0.8250	92.53	91.62	90.61	89.64	88.67
19	.51	.56	.51	.48	.48	49	.55	.64	.64	.67	.70
18	.54	.59	.54	.51	.52	48	.58	.67	.67	.70	.73
17	.57	.62	.57	.54	.55	47	.61	.70	.70	.73	.76
16	.60	.65	.60	.58	.58	46	.64	.73	.73	.76	.79
15	.63	.68	.64	.61	.61	45	.66	.76	.76	.79	.83
14	.66	.71	.67	.64	.65	44	.69	.79	.79	.82	.86
13	.69	.74	.70	.67	.68	43	.72	.82	.82	.85	.89
12	.72	.77	.73	.70	.71	42	.75	.85	.85	.88	.92
11	.75	.80	.76	.74	.74	41	.78	.87	.88	.91	.95
10	.78	.83	.79	.77	.77	40	.80	.90	.91	.94	.98
09	.81	.86	.82	.80	.81	39	.83	.93	.94	.97	89.01
08	.84	.89	.85	.83	.84	38	.86	.96	.97	90.00	.04
07	.87	.93	.88	.86	.87	37	.89	.99	91.00	.03	.07
06	.90	.96	.92	.89	.90	36	.92	92.02	.03	.06	.10
05	.93	.99	.95	.93	.94	35	.94	.05	.06	.09	.13
04	.96	90.02	.98	.96	.97	34	.97	.08	.09	.12	.16
03	.99	.05	89.01	.99	87.00	33	93.00	.10	.12	.15	.20
02	91.02	.08	.04	88.02	.03	32	.03	.13	.15	.18	.23
01	.05	.11	.07	.05	.06	31	.05	.16	.18	.21	.26
00	.08	.14	.10	.08	.10	30	.08	.19	.21	.24	.29
08299	.11	.17	.13	.12	.13	29	.11	.22	.23	.27	.32
98	.14	.20	.16	.15	.16	28	.14	.25	.26	.30	.35
97	.17	.23	.19	.18	.19	27	.16	.28	.29	.33	.38
96	.20	.26	.22	.21	.22	26	.19	.31	.32	.36	.41
95	.23	.29	.25	.24	.25	25	.22	.33	.35	.39	.44
94	.26	.32	.28	.27	.29	24	.25	.36	.38	.42	.47
93	.28	.35	.31	.30	.32	23	.27	.39	.41	.45	.50
92	.31	.38	.35	.34	.35	22	.30	.42	.44	.48	.53
91	.34	.41	.38	.37	.38	21	.33	.45	.47	.51	.56
90	.37	.44	.41	.40	.41	20	.36	.48	.50	.54	.59
89	.40	.47	.44	.43	.44	19	.38	.50	.52	.57	.62
88	.43	.50	.47	.46	.48	18	.41	.53	.55	.60	.65
87	.46	.53	.50	.49	.51	17	.44	.56	.58	.63	.68
86	.49	.56	.53	.52	.54	16	.47	.59	.61	.66	.71
85	.52	.59	.56	.55	.57	15	.49	.62	.64	.69	.74
84	.55	.62	.59	.59	.60	14	.52	.65	.67	.72	.77
83	.58	.65	.62	.62	.63	13	.55	.67	.70	.75	.80
82	.60	.67	.65	.65	.67	12	.58	.70	.73	.78	.84
81	.63	.70	.68	.68	.70	11	.60	.73	.76	.81	.87
80	.66	.73	.71	.71	.73	10	.63	.76	.79	.84	.90
79	.69	.76	.74	.74	.76	09	.66	.79	.81	.87	.93
78	.72	.79	.77	.77	.79	08	.68	.81	.84	.90	.96
77	.75	.82	.80	.81	.83	07	.71	.84	.87	.93	.99
76	.78	.85	.83	.84	.86	06	.74	.87	.90	.96	90.02
75	.81	.88	.87	.87	.89	05	.76	.90	.93	.99	.05
74	.84	.91	.90	.90	.92	04	.79	.92	.96	91.01	.08
73	.87	.94	.93	.93	.95	03	.82	.95	.99	.04	.11
72	.90	.97	.96	.96	.98	02	.84	.98	92.02	.07	.14
71	.92	91.00	.99	.99	88.02	01	.87	93.01	.05	.10	.17
70	.95	.03	90.02	89.02	.05	00	.90	.04	.07	.13	.20
69	.98	.06	.05	.06	.08	0.8199	.92	.06	.10	.16	.23
68	92.01	.09	.08	.09	.11	98	.95	.09	.13	.19	.26
67	.04	.12	.11	.12	.14	97	.98	.12	.16	.22	.29
66	.07	.15	.14	.15	.17	96	94.01	.14	.19	.25	.32
65	.10	.18	.17	.18	.20	95	.03	.17	.22	.27	.35
64	.13	.21	.20	.21	.23	94	.06	.20	.24	.30	.38
63	.15	.24	.23	.24	.26	93	.08	.23	.27	.33	.40
62	.18	.27	.26	.27	.30	92	.11	.25	.30	.36	.43
61	.21	.29	.29	.30	.33	91	.14	.28	.33	.39	.46
60	.24	.32	.32	.33	.36	90	.16	.31	.36	.42	.49
59	.27	.35	.35	.36	.39	89	.19	.34	.39	.45	.52
58	.30	.38	.38	.39	.42	88	.22	.36	.41	.48	.55
57	.33	.41	.41	.42	.45	87	.24	.39	.44	.51	.58
56	.35	.44	.44	.45	.48	86	.27	.42	.47	.53	.61
55	.38	.47	.47	.48	.51	85	.30	.44	.50	.56	.64
54	.41	.50	.50	.51	.55	84	.32	.47	.53	.59	.67
53	.44	.53	.53	.54	.58	83	.35	.50	.56	.62	.70
52	.47	.56	.56	.57	.61	82	.38	.53	.58	.65	.73
51	.50	.59	.59	.61	.64	81	.40	.55	.61	.68	.76
50	.53	.62	.61	.64	.67	80	.43	.58	.64	.71	.79

43.021 *Percentages by vol. at 15.56°C (60°F) of ethyl alcohol corresponding to apparent specific gravities at various temps*—Continued.

APPARENT SPECIFIC GRAVITY	15.56/15.56	20/20	25/25	30/30	35/35
0.8180	94.43	93.58	92.64	91.71	90.79
79	.46	.61	.67	.74	.82
78	.48	.64	.70	.77	.85
77	.51	.66	.72	.79	.88
76	.53	.69	.75	.82	.91
75	.56	.72	.78	.85	.94
74	.59	.74	.81	.88	.97
73	.61	.77	.84	.91	91.00
72	.64	.80	.86	.94	.03
71	.66	.82	.89	.97	.06
70	.69	.85	.92	92.00	.09
69	.72	.88	.95	.03	.12
68	.74	.90	.97	.05	.14
67	.77	.93	93.00	.08	.17
66	.79	.96	.03	.11	.20
65	.82	.98	.06	.14	.23
64	.84	94.01	.09	.17	.26
63	.87	.04	.11	.20	.29
62	.90	.06	.14	.22	.32
61	.92	.09	.17	.25	.35
60	.95	.12	.20	.28	.38
59	.97	.14	.22	.31	.40
58	95.00	.17	.25	.34	.43
57	.03	.20	.28	.36	.46
56	.05	.22	.30	.39	.49
55	.08	.25	.33	.42	.52
54	.10	.28	.36	.45	.55
53	.13	.30	.39	.48	.58
52	.15	.33	.41	.51	.61
51	.18	.36	.44	.54	.64
50	.20	.38	.47	.56	.66
49	.23	.41	.50	.59	.69
48	.25	.44	.52	.62	.72
47	.28	.46	.55	.65	.75
46	.30	.49	.58	.68	.78
45	.33	.51	.60	.70	.81
44	.36	.54	.63	.73	.84
43	.38	.57	.66	.76	.87
42	.41	.59	.69	.79	.90
41	.43	.62	.71	.82	.92
40	.46	.64	.74	.84	.95
39	.48	.67	.77	.87	.98
38	.51	.70	.79	.90	92.01
37	.53	.72	.82	.93	.04
36	.56	.75	.85	.96	.07
35	.58	.77	.87	.98	.10
34	.61	.80	.90	93.01	.13
33	.63	.83	.93	.04	.15
32	.66	.85	.95	.07	.18
31	.68	.88	.98	.09	.21
30	.71	.90	94.01	.12	.24
29	.73	.93	.03	.15	.27
28	.76	.95	.06	.18	.30
27	.78	.98	.09	.20	.33
26	.81	95.01	.11	.23	.35
25	.83	.03	.14	.26	.38
24	.86	.06	.17	.29	.41
23	.88	.08	.19	.31	.44
22	.91	.11	.22	.34	.47
21	.93	.14	.24	.37	.50
20	.96	.16	.27	.40	.53
19	.98	.19	.30	.42	.55
18	96.01	.21	.32	.45	.58
17	.03	.24	.35	.48	.61
16	.06	.26	.38	.51	.64
15	.08	.29	.40	.53	.66
14	.10	.32	.43	.56	.69
13	.13	.34	.46	.59	.72
12	.15	.37	.48	.61	.75
11	.18	.39	.51	.64	.78
10	.20	.42	.53	.67	.80

APPARENT SPECIFIC GRAVITY	15.56/15.56	20/20	25/25	30/30	35/35
0.8110	96.20	95.42	94.53	93.67	92.80
09	.23	.44	.56	.69	.83
08	.25	.47	.59	.72	.86
07	.28	.49	.61	.75	.89
06	.30	.52	.64	.77	.92
05	.32	.54	.66	.80	.94
04	.35	.57	.69	.83	.97
03	.37	.59	.72	.85	93.00
02	.40	.62	.74	.88	.03
01	.42	.64	.77	.91	.05
00	.45	.67	.79	.94	.08
0.8099	.47	.69	.82	.96	.11
98	.50	.72	.85	.99	.14
97	.52	.74	.87	94.02	.16
96	.54	.77	.90	.04	.19
95	.57	.79	.92	.07	.22
94	.59	.82	.95	.10	.25
93	.61	.84	.98	.12	.27
92	.64	.87	95.00	.15	.30
91	.66	.89	.03	.17	.33
90	.69	.92	.05	.20	.36
89	.71	.94	.08	.23	.38
88	.73	.97	.10	.25	.41
87	.76	.99	.13	.28	.44
86	.78	96.02	.16	.31	.46
85	.81	.04	.18	.33	.49
84	.83	.07	.21	.36	.52
83	.85	.09	.23	.39	.55
82	.88	.11	.26	.41	.57
81	.90	.14	.28	.44	.60
80	.93	.16	.31	.47	.63
79	.95	.19	.33	.49	.65
78	.97	.21	.36	.52	.68
77	97.00	.24	.39	.54	.71
76	.02	.26	.41	.57	.73
75	.04	.29	.44	.60	.76
74	.07	.31	.46	.62	.79
73	.09	.33	.49	.65	.81
72	.11	.36	.51	.67	.84
71	.14	.38	.54	.70	.87
70	.16	.41	.56	.73	.90
69	.18	.43	.59	.75	.92
68	.21	.46	.61	.78	.95
67	.23	.48	.64	.80	.98
66	.25	.50	.66	.83	94.00
65	.28	.53	.69	.86	.03
64	.30	.55	.71	.88	.06
63	.32	.58	.74	.91	.08
62	.35	.60	.76	.93	.11
61	.37	.63	.79	.96	.14
60	.39	.65	.81	.99	.16
59	.42	.67	.84	95.01	.19
58	.44	.70	.86	.04	.22
57	.46	.72	.89	.06	.24
56	.49	.75	.91	.09	.27
55	.51	.77	.94	.11	.30
54	.53	.79	.96	.14	.32
53	.55	.82	.99	.17	.35
52	.58	.84	96.01	.19	.38
51	.60	.86	.04	.22	.40
50	.62	.89	.06	.24	.43
49	.64	.91	.09	.27	.45
48	.67	.94	.11	.29	.48
47	.69	.96	.14	.32	.51
46	.71	.98	.16	.34	.53
45	.73	97.01	.19	.37	.56
44	.76	.03	.21	.40	.59
43	.78	.05	.24	.42	.61
42	.80	.08	.26	.45	.64
41	.82	.10	.28	.47	.66
40	.85	.12	.31	.50	.69

Percentages by vol. at 15.56°C (60°F) of ethyl alcohol corresponding to apparent specific gravities at various temps—Continued. **43.021**

Apparent specific gravity	15.56/15.56	20/20	25/25	30/30	35/35	Apparent specific gravity	15.56/15.56	20/20	25/25	30/30	35/35
0.8040	97.85	97.12	96.31	95.50	94.69	0.7970	99.33	98.68	97.95	97.21	96.46
39	.87	.15	.33	.52	.72	69	.35	.70	.97	.23	.48
38	.89	.17	.36	.55	.74	68	.37	.72	.99	.25	.51
37	.91	.19	.38	.57	.77	67	.39	.75	98.02	.28	.53
36	.94	.22	.41	.60	.79	66	.42	.77	.04	.30	.56
35	.96	.24	.43	.62	.82	65	.44	.79	.06	.32	.58
34	.98	.26	.46	.65	.85	64	.46	.81	.08	.35	.60
33	98.00	.29	.48	.67	.87	63	.48	.83	.10	.37	.63
32	.03	.31	.50	.70	.90	62	.50	.85	.12	.39	.65
31	.05	.33	.53	.72	.92	61	.52	.87	.15	.42	.68
30	.07	.36	.55	.75	.95	60	.54	.89	.17	.44	.70
29	.09	.38	58	.77	.98	59	.56	.91	.19	.46	.72
28	.11	.40	.60	.80	95.00	58	.58	.93	.21	.49	.75
27	.14	.43	.62	.82	.03	57	.60	.95	.23	.51	.77
26	.16	.45	.65	.85	.05	56	.61	.97	.26	.53	.80
25	.18	.47	.67	.87	.08	55	.63	99.00	.28	.56	.82
24	.20	.49	.70	.90	.11	54	.65	.02	.30	.58	.84
23	.22	.52	.72	.92	13	53	.67	.04	.32	.60	.87
22	.25	.54	.74	.95	.16	52	.69	.06	.34	.62	.89
21	.27	.56	.77	.97	.18	51	.71	.08	.36	.65	.92
20	.29	.59	.79	96.00	.21	50	.73	.10	.39	.67	.94
19	.31	.61	.82	.02	.23	49	.75	.12	.41	.69	.96
18	.33	.63	.84	.05	.26	48	.77	.14	.43	.71	.99
17	.35	.66	.86	.07	.28	47	.79	.16	.45	.74	97.01
16	.38	.68	.89	.10	.31	46	.81	.18	.47	.76	.04
15	.40	.70	.91	.12	.34	45	.83	.20	.49	.78	.06
14	.42	.72	.94	.15	.36	44	.85	.22	.51	.80	.08
13	.44	.75	.96	.17	.39	43	.87	.24	.54	.83	.11
12	.46	.77	.98	.20	.41	42	.89	.26	.56	.85	.13
11	.48	.79	97.01	.22	.44	41	.91	.28	.58	.87	.15
10	.50	.81	.03	.25	.46	40	.93	.30	.60	.89	.18
09	.53	.84	.05	.27	.49	39	.95	.32	.62	.92	.20
08	.55	.86	.08	.29	.52	38	.97	.34	.64	.94	.22
07	.57	.88	.10	.32	.54	37	.99	.36	.66	.96	.25
06	.59	.90	.12	.34	.57	36	100.00	.38	.68	.98	.27
05	.61	.92	.15	.37	.59	35		.40	.70	98.01	.29
04	.63	.95	.17	.39	.62	34		.42	.73	.03	.32
03	.65	.97	.19	.42	.64	33		.44	.75	.05	.34
02	.67	.99	.22	.44	.67	32		.46	.77	.07	.36
01	.70	98.01	.24	.47	.69	31		.48	.79	.09	.39
00	.72	.03	.26	.49	.72	30		.50	.81	.12	.41
0.7999	.74	.06	.29	.51	.74	29		.52	.83	.14	.43
98	.76	.08	.31	.54	.77	28		.54	.85	.16	.46
97	.78	.10	.33	.56	.79	27		.56	.87	.18	.48
96	.80	.12	.36	.59	.82	26		.58	.89	.20	.50
95	.82	.14	.38	.61	.84	25		.60	.91	.23	.52
94	.84	.17	.40	.63	.87	24		.62	.93	.25	.55
93	.86	.19	.43	.66	.89	23		.64	.96	.27	.57
92	.88	.21	.45	.68	.92	22		.66	.98	.29	.59
91	.90	.23	.47	.71	.94	21		.68	99.00	.31	.62
90	.92	.26	.50	.73	.97	20		.70	.02	.33	.64
89	.95	.28	.52	.75	.99	19		.72	.04	.36	.66
88	.97	.30	.54	.78	96.02	18		.74	.06	.38	.68
87	.99	.32	.57	.80	.04	17		.76	.08	.40	.71
86	99.01	.34	.59	.83	.07	16		.78	.10	.42	.73
85	.03	.36	.61	.85	.09	15		.80	.12	.44	.75
84	.05	.39	.63	.87	.12	14		.82	.14	.46	.77
83	.07	.41	.66	.90	.14	13		.84	.16	.48	.80
82	.09	.43	.68	.92	.16	12		.86	.18	.51	.82
81	.11	.45	.70	.95	.19	11		.88	.20	.53	.84
80	.13	.47	.72	.97	.21	10		.90	.22	.55	.86
79	.15	.49	.75	97.00	.24	09		.92	.24	.57	.89
78	.17	.51	.77	.02	.26	08		.94	.27	.59	.91
77	.19	.54	.79	.04	.29	07		.96	.29	.61	.93
76	.21	.56	.81	.07	.31	06		.98	.31	.63	.95
75	.23	.58	.84	.09	.34	05		100.00	.33	.66	.98
74	.25	.60	.86	.11	.36	04			.35	.68	98.00
73	.27	.62	.88	.14	.38	03			.37	.70	.02
72	.29	.64	.90	.16	.41	02			.39	.72	.04
71	.31	.66	.93	.18	.43	01			.41	.74	.07
70	.33	.68	.95	.21	.46	00			.43	.76	.09

43.021 *Percentages by vol. at 15.56°C (60°F) of ethyl alcohol corresponding to apparent specific gravities at various temps*—Concluded.

APPARENT SPECIFIC GRAVITY	25/25	30/30	35/35
0.7900	99.43	98.76	98.09
.7899	.45	.78	.11
98	.47	.80	.13
97	.49	.82	.15
96	.51	.84	.18
95	.53	.87	.20
94	.55	.89	.22
93	.57	.91	.24
92	.59	.93	.26
91	.61	.95	.29
90	.63	.97	.31
89	.65	.99	.33
88	.67	99.01	.35
87	.69	.03	.37
86	.71	.05	.40
85	.73	.07	.42
84	.75	.09	.44
83	.77	.12	.46
82	.79	.14	.48
81	.81	.16	.50
80	.83	.18	.53
79	.85	.20	.55
78	.86	.22	.57
77	.88	.24	.59
76	.90	.26	.61
75	.92	.28	.63
74	.94	.30	.65
73	.96	.32	.67
72	.98	.34	.70
71	100.00	.36	.72
70		.38	.74
69		.40	.76
68		.42	.78
67		.44	.80
66		.46	.82
65		.48	.84
64		.50	.86
63		.52	.89
62		.54	.91
61		.56	.93
60		.58	.95
59		.60	.97
58		.62	.99
57		.64	99.01
56		.66	.03
55		.68	.05
54		.70	.07
53		.72	.09
52		.74	.12
51		.76	.14
50		.78	.16
49		.80	.18
48		.72	.20
47		.84	.22
46		.86	.24
45		.88	.26
44		.90	.28
43		.92	.30
42		.94	.32
41		.96	.34
40		.98	.36
39		100.00	.38
38			.40
37			.42
36			.44
35			.46
34			.48
33			.50
32			.52
31			.54
30			.56

APPARENT SPECIFIC GRAVITY	35/35
0.7830	99.56
29	.58
28	.60
27	.62
26	.64
25	.66
24	.68
23	.70
22	.72
21	.74
20	.76
19	.78
18	.80
17	.82
16	.84
15	.86
14	.88
13	.90
12	.92
11	.94
09	.08
08	100.00

Alcohol table for calcg percentages of alcohol by vol. at 15.56°C (60°F) in mixts of ethyl alcohol and H_2O from their Zeiss immersion refractometer readings and indices of refraction at 17.5–25°C[a] 43.022

SCALE READING[b]	INDEX OF REFRACTION	17.5° C	18° C	19° C	20° C	21° C	22° C	23° C	24° C	25° C
13.2	1.33250	....	....	....	....	....	....	....	....	0.00
13.4	3257	....	....	....	....	....	....	....	....	0.18
13.6	3265	....	....	....	....	....	....	....	0.14	0.35
13.8	3273	....	....	....	....	....	....	0.10	0.31	0.53
14.0	3281	....	....	....	....	....	0.08	0.28	0.49	0.70
14.2	3288	....	....	....	....	0.04	0.24	0.45	0.67	0.88
14.4	3296	....	....	....	....	0.21	0.41	0.63	0.84	1.06
14.6	3304	....	....	....	0.16	0.38	0.59	0.80	1.02	1.24
14.8	3312	....	....	0.14	0.34	0.55	0.77	0.98	1.19	1.40
15.0	3319	0.00	0.10	0.31	0.52	0.73	0.94	1.16	1.36	1.55
15.2	3327	0.17	0.27	0.48	0.69	0.91	1.12	1.32	1.51	1.71
15.4	3335	0.34	0.44	0.65	0.85	1.07	1.29	1.47	1.66	1.86
15.6	3343	0.51	0.60	0.82	1.03	1.24	1.44	1.62	1.82	2.01
15.8	3350	0.68	0.78	0.99	1.21	1.40	1.60	1.77	1.97	2.17
16.0	3358	0.84	0.94	1.17	1.36	1.55	1.75	1.92	2.12	2.33
16.2	3366	1.02	1.12	1.32	1.51	1.70	1.90	2.08	2.27	2.48
16.4	3374	1.18	1.29	1.47	1.66	1.85	2.05	2.24	2.43	2.62
16.6	3381	1.34	1.43	1.62	1.81	2.00	2.20	2.39	2.57	2.77
16.8	3389	1.49	1.57	1.77	1.96	2.15	2.35	2.53	2.72	2.92
17.0	3397	1.63	1.72	1.92	2.11	2.30	2.50	2.69	2.87	3.06
17.2	3405	1.77	1.87	2.06	2.26	2.45	2.65	2.82	3.02	3.21
17.4	3412	1.92	2.01	2.21	2.41	2.59	2.79	2.97	3.17	3.36
17.6	3420	2.07	2.16	2.36	2.56	2.74	2.94	3.12	3.32	3.51
17.8	3428	2.21	2.31	2.51	2.70	2.89	3.09	3.27	3.46	3.66
18.0	3435	2.36	2.45	2.66	2.85	3.04	3.23	3.42	3.61	3.81
18.2	3443	2.50	2.60	2.81	3.00	3.19	3.37	3.57	3.76	3.96
18.4	3451	2.65	2.75	2.96	3.15	3.34	3.52	3.71	3.91	4.11
18.6	3459	2.80	2.90	3.10	3.30	3.48	3.66	3.86	4.06	4.26
18.8	3466	2.95	3.05	3.25	3.45	3.63	3.81	4.01	4.21	4.41
19.0	3474	3.10	3.19	3.40	3.59	3.77	3.96	4.16	4.36	4.56
19.2	3482	3.25	3.34	3.55	3.73	3.92	4.11	4.31	4.51	4.70
19.4	3489	3.39	3.48	3.70	3.88	4.07	4.26	4.46	4.65	4.85
19.6	3497	3.53	3.63	3.84	4.03	4.22	4.41	4.61	4.80	5.00
19.8	3505	3.68	3.78	3.98	4.17	4.37	4.56	4.75	4.95	5.15
20.0	3513	3.83	3.93	4.13	4.32	4.52	4.72	4.90	5.10	5.29
20.2	3520	3.97	4.07	4.27	4.47	4.66	4.87	5.05	5.24	5.44
20.4	3528	4.12	4.22	4.42	4.61	4.82	5.01	5.20	5.38	5.58
20.6	3536	4.26	4.36	4.56	4.75	4.96	5.15	5.34	5.52	5.72
20.8	3543	4.41	4.51	4.70	4.90	5.10	5.29	5.48	5.67	5.87
21.0	3551	4.56	4.65	4.85	5.04	5.24	5.44	5.62	5.82	6.02
21.2	3559	4.70	4.80	4.99	5.19	5.39	5.58	5.77	5.96	6.16
21.4	3566	4.84	4.94	5.14	5.33	5.53	5.72	5.91	6.11	6.30
21.6	3574	4.99	5.09	5.28	5.47	5.67	5.87	6.06	6.25	6.44
21.8	3582	5.13	5.23	5.43	5.61	5.82	6.01	6.20	6.39	6.59

[a] Rearranged from table of B. H. St. John, which is based upon data of Doroschevskii and Dvorzhanchik. *J. Russ. Phys. Chem. Soc.*, 40, 101 (1908). Scale readings were converted into refractive indices by using formula $n_D = 1.327338 + 0.00039347X - 0.00000020446X^2$.

[b] Scale readings refer only to scale of arbitrary units proposed by Pulfrich, *Z. angew. Chem.*, p. 1168, 1899. According to this scale, 14.5 = 1.33300, 50.0 = 1.34650, and 100.0 = 1.36464. If immersion refractometer used is calibrated to another arbitrary scale, readings must be converted into refractive indices before table is used to det. % alcohol.

43.022 *Alcohol table*—Continued.

SCALE READING	INDEX OF REFRACTION	17.5° C	18° C	19° C	20° C	21° C	22° C	23° C	24° C	25° C
22.0	1.33590	5.27	5.37	5.57	5.76	5.96	6.15	6.34	6.54	6.73
22.2	3597	5.41	5.51	5.71	5.90	6.11	6.29	6.49	6.68	6.87
22.4	3605	5.56	5.65	5.85	6.05	6.25	6.43	6.63	6.82	7.01
22.6	3613	5.70	5.80	6.00	6.19	6.39	6.57	6.77	6.96	7.16
22.8	3620	5.85	5.94	6.14	6.33	6.53	6.71	6.91	7.10	7.31
23.0	3628	5.99	6.08	6.28	6.47	6.67	6.86	7.06	7.24	7.45
23.2	3636	6.13	6.22	6.42	6.61	6.81	7.00	7.20	7.39	7.59
23.4	3643	6.27	6.36	6.56	6.75	6.95	7.14	7.34	7.53	7.73
23.6	3651	6.41	6.50	6.70	6.90	7.09	7.28	7.48	7.67	7.87
23.8	3659	6.55	6.64	6.85	7.04	7.23	7.42	7.62	7.81	8.00
24.0	3666	6.69	6.78	6.99	7.18	7.38	7.56	7.76	7.95	8.14
24.2	3674	6.83	6.92	7.13	7.32	7.52	7.70	7.90	8.09	8.28
24.4	3682	6.97	7.06	7.27	7.46	7.66	7.84	8.04	8.23	8.42
24.6	3689	7.11	7.20	7.41	7.60	7.80	7.98	8.17	8.37	8.55
24.8	3697	7.25	7.35	7.55	7.74	7.93	8.12	8.31	8.51	8.69
25.0	3705	7.39	7.49	7.68	7.88	8.06	8.26	8.45	8.64	8.84
25.2	3712	7.53	7.63	7.82	8.01	8.20	8.40	8.59	8.78	8.98
25.4	3720	7.66	7.76	7.95	8.14	8.34	8.54	8.73	8.92	9.12
25.6	3728	7.80	7.90	8.09	8.28	8.48	8.68	8.86	9.06	9.26
25.8	3735	7.94	8.03	8.22	8.42	8.62	8.82	9.00	9.20	9.39
26.0	3743	8.07	8.16	8.36	8.55	8.75	8.95	9.14	9.34	9.53
26.2	3751	8.21	8.30	8.50	8.69	8.89	9.09	9.28	9.48	9.67
26.4	3758	8.34	8.44	8.63	8.82	9.03	9.22	9.42	9.61	9.81
26.6	3766	8.48	8.57	8.77	8.96	9.16	9.36	9.55	9.75	9.95
26 8	3774	8.62	8.71	8.91	9.10	9.30	9.49	9.69	9.89	10.09
27.0	3781	8.75	8.85	9.05	9.23	9.44	9.63	9.83	10.03	10.23
27.2	3789	8.89	8.98	9.18	9.37	9.58	9.76	9.97	10.17	10.37
27.4	3796	9.02	9.12	9.32	9.51	9.71	9.90	10.10	10.31	10.51
27.6	3804	9.16	9.26	9.45	9.65	9.85	10.03	10 24	10.45	10.65
27.8	3812	9.29	9.39	9.59	9.79	9.98	10.17	10.38	10.58	10.79
28.0	3820	9.43	9.53	9.72	9.92	10.12	10.31	10.51	10.72	10.93
28.2	3827	9.57	9.66	9.86	10.06	10.25	10.45	10.65	10.86	11.06
28.4	3835	9.70	9.80	9.99	10.19	10.39	10.59	10.79	11.00	11.20
28.6	3842	9.84	9.93	10.13	10.32	10.52	10.72	10.93	11.13	11.33
28.8	3850	9.97	10.07	10.26	10.46	10.66	10.86	11 06	11.27	11.47
29.0	3858	10.10	10.19	10.40	10.59	10.79	11.00	11.20	11.40	11.61
29.2	3865	10.24	10.33	10.52	10.73	10.93	11.13	11.33	11.54	11.75
29.4	3873	10.36	10.46	10.66	10.86	11.06	11.27	11.47	11.67	11.88
29.6	3881	10.50	10.59	10.79	10.99	11.20	11.39	11.60	11.81	12.01
29.8	3888	10.63	10.72	10.93	11.12	11.33	11.53	11.74	11.94	12.15
30.0	3896	10.76	10.86	11 05	11.26	11.46	11.66	11.87	12.08	12.29
30.2	3904	10.89	10.99	11.18	11.38	11.59	11.79	12.00	12.21	12.42
30.4	3911	11.02	11.12	11.31	11.51	11.72	11.93	12.13	12.34	12.56
30.6	3919	11.15	11.25	11.44	11.64	11.85	12.06	12.27	12.48	12.70
30.8	3926	11.28	11.38	11.58	11.78	11.99	12.19	12.40	12.61	12.84

Alcohol table—Continued. 43.022

SCALE READING	INDEX OF REFRACTION	17.5° C	18° C	19° C	20° C	21° C	22° C	23°C	24° C	25° C
31.0	1.33934	11.41	11.51	11.71	11.91	12.12	12.32	12.54	12.75	12.97
31.2	3942	11.54	11.64	11.84	12.04	12.25	12.46	12.67	12.89	13.11
31.4	3949	11.66	11.77	11.97	12.17	12.38	12.59	12.81	13.02	13.24
31.6	3957	11.79	11.90	12.10	12.30	12.51	12.72	12.94	13.15	13.37
31.8	3964	11.92	12.03	12.23	12.43	12.64	12.85	13.07	13.29	13.51
32.0	3972	12.05	12.15	12.36	12.57	12.78	12.99	13.20	13.42	13.64
32.2	3980	12.18	12.28	12.49	12.70	12.91	13.12	13.34	13.55	13.77
32.4	3987	12.31	12.40	12.62	12.83	13.04	13.25	13.47	13.69	13.91
32.6	3995	12.43	12.54	12.75	12.96	13.17	13.38	13.60	13.82	14.04
32.8	4002	12.56	12.67	12.88	13.09	13.30	13.51	13.73	13.95	14.17
33.0	4010	12.69	12.79	13.01	13.22	13.43	13.64	13.86	14.09	14.31
33.2	4018	12.82	12.92	13.13	13.35	13.56	13.78	13.99	14.22	14.44
33.4	4025	12.95	13.05	13.26	13.48	13.69	13.91	14.13	14.35	14.58
33.6	4033	13.08	13.18	13.39	13.61	13.82	14.04	14.26	14.48	14.71
33.8	4040	13.20	13.30	13.52	13.74	13.95	14.17	14.39	14.62	14.85
34.0	4048	13.33	13.43	13.64	13.86	14.08	14.30	14.52	14.75	14.98
34.2	4056	13.45	13.56	13.77	13.99	14.21	14.43	14.65	14.88	15.11
34.4	4063	13.58	13.68	13.90	14.12	14.34	14.57	14.78	15.01	15.25
34.6	4071	13.70	13.81	14.02	14.25	14.47	14.70	14.91	15.14	15.38
34.8	4078	13.83	13.94	14.14	14.37	14.59	14.83	15.05	15.28	15.51
35.0	4086	13.96	14.06	14.27	14.50	14.72	14.96	15.18	15.41	15.65
35.2	4094	14.08	14.19	14.39	14.62	14.85	15.09	15.31	15.54	15.78
35.4	4101	14.21	14.31	14.52	14.75	14.97	15.22	15.44	15.67	15.91
35.6	4109	14.33	14.44	14.65	14.87	15.10	15.34	15.56	15.80	16.05
35.8	4116	14.46	14.56	14.78	15.00	15.23	15.47	15.69	15.93	16.18
36.0	4124	14.58	14.69	14.90	15.13	15.35	15.59	15.82	16.06	16.31
36.2	4131	14.71	14.81	15.03	15.25	15.48	15.72	15.95	16.19	16.44
36.4	4139	14.83	14.94	15.16	15.38	15.61	15.85	16.08	16.32	16.56
36.6	4146	14.96	15.06	15.28	15.51	15.73	15.97	16.21	16.45	16.69
36.8	4154	15.08	15.19	15.41	15.63	15.86	16.10	16.34	16.58	16.82
37.0	4162	15.20	15.31	15.53	15.76	15.99	16.23	16.47	16.71	16.95
37.2	4169	15.33	15.44	15.66	15.89	16.11	16.35	16.60	16.84	17.08
37.4	4177	15.45	15.56	15.79	16.01	16.24	16.48	16.72	16.97	17.21
37.6	4184	15.57	15.69	15.91	16.14	16.37	16.61	16.85	17.09	17.34
37.8	4192	15.70	15.81	16.04	16.26	16.49	16.73	16.98	17.22	17.46
38.0	4199	15.82	15.94	16.16	16.39	16.62	16.86	17.11	17.35	17.59
38.2	4207	15.94	16.06	16.29	16.51	16.75	16.99	17.23	17.47	17.72
38.4	4215	16.07	16.18	16.41	16.64	16.87	17.11	17.36	17.60	17.85
38.6	4222	16.19	16.31	16.53	16.76	17.00	17.24	17.48	17.73	17.97
38.8	4230	16.31	16.43	16.66	16.89	17.13	17.36	17.61	17.85	18.10
39.0	4237	16.44	16.55	16.78	17.01	17.25	17.49	17.74	17.98	18.23
39.2	4245	16.56	16.67	16.91	17.14	17.38	17.62	17.86	18.11	18.35
39.4	4252	16.68	16.80	17.03	17.26	17.50	17.74	17.99	18.23	18.48
39.6	4260	16.80	16.92	17.15	17.39	17.63	17.87	18.11	18.36	18.61
39.8	4267	16.93	17.04	17.28	17.51	17.75	17.99	18.24	18.48	18.73

43.022 *Alcohol table*—Continued.

SCALE READING	INDEX OF REFRACTION	17.5° C	18° C	19° C	20° C	21° C	22° C	23° C	24° C	25° C
40.0	1.34275	17.05	17.16	17.40	17.63	17.88	18.12	18.36	18.61	18.86
40.2	4282	17 17	17.29	17.52	17 76	18.00	18.24	18.49	18.74	18.99
40.4	4290	17.29	17.41	17.64	17.88	18.12	18.37	18.61	18.86	19.11
40.6	4298	17 41	17.53	17.77	18.01	18.25	18.49	18.74	18.99	19.24
40.8	4305	17 54	17.65	17.89	18.13	18.37	18.61	18.86	19.11	19.37
41.0	4313	17.66	17 77	18.01	18 25	18.49	18.74	18.99	19 24	19.49
41.2	4320	17.78	17.90	18.13	18.37	18.62	18.86	19.11	19 36	19.62
41.4	4328	17.90	18.03	18.26	18.50	18.74	18.99	19.24	19 49	19.75
41.6	4335	18.02	18 14	18 38	18.62	18.86	19.11	19.36	19.61	19.87
41.8	4343	18.14	18.26	18 50	18.74	18 99	19 23	19.48	19.74	20.00
42.0	4350	18 27	18.38	18 62	18.87	19.11	19.36	19.61	19.86	20.13
42.2	4358	18 39	18.50	18.74	18.99	19.23	19.48	19.73	19.99	20.25
42.4	4365	18 51	18 62	18.87	19.11	19.36	19.60	19.86	20.11	20.38
42.6	4373	18.63	18.75	18.99	19.23	19.48	19.72	19.98	20.24	20.50
42.8	4380	18 75	18.87	19.11	19 36	19.60	19.85	20.10	20.36	20.63
43.0	4388	18.87	18 99	19 23	19.48	19.72	19.97	20.23	20.49	20.75
43.2	4395	18.99	19.11	19 35	19.60	19.85	20.09	20.35	20.61	20.88
43.4	4403	19.11	19.23	19 47	19.72	19.97	20.21	20.47	20.74	21.01
43.6	4410	19.23	19.35	19 59	19 85	20.09	20.34	20.60	20.86	21.13
43.8	4418	19.35	19.47	19 72	19.97	20.21	20.46	20 72	20.99	21.25
44.0	4426	19.46	19.59	19.84	20.09	20.34	20.58	20.84	21.11	21.38
44 2	4433	19.58	19.71	19.96	20.21	20.46	20.71	20 96	21.23	21.50
44.4	4440	19.70	19.83	20.08	20.33	20.58	20.83	21.09	21.36	21.63
44 6	4448	19.82	19 95	20.20	20.45	20.70	20.95	21.21	21.48	21.75
44 8	4456	19 94	20.07	20.32	20.58	20.82	21.07	21.33	21 60	21.88
45.0	4463	20.06	20 18	20.44	20.70	20.95	21.19	21.45	21.73	22.00
45.2	4470	20 18	20 30	20 56	20.82	21.07	21.31	21.58	21.85	22.13
45.4	4478	20.29	20.42	20 68	20.94	21.19	21.43	21.70	21.98	22.25
45.6	4486	20 41	20.54	20.80	21.06	21.31	21.55	21.82	22.10	22.38
45 8	4493	20 53	20 66	20 92	21 18	21.43	21.67	21.94	22.23	22.51
46.0	4500	20 65	20.78	21.04	21 30	21.54	21.79	22.07	22.35	22.64
46.2	4508	20.76	20 89	21 16	21 42	21.66	21.91	22.19	22.48	22.76
46.4	4516	20 88	21 01	21 28	21.54	21.78	22.03	22.32	22.61	22.89
46.6	4523	21.00	21 13	21.40	21 66	21.90	22.16	22.44	22.73	23.02
46.8	4530	21.12	21.25	21 52	21.78	22.02	22.28	22.57	22.86	23.15
47.0	4538	21.24	21.37	21.64	21.90	22.15	22.41	22.69	22.99	23.28
47.2	4545	21.36	21.49	21.76	22.02	22.27	22.53	22.82	23.12	23.41
47.4	4553	21.48	21.61	21.88	22.15	22.39	22.66	22.94	23.24	23.54
47.6	4560	21.60	21.73	22.00	22.27	22.51	22.78	23.07	23.37	23.67
47.8	4568	21.72	21.85	22.12	22.39	22.64	22.91	23.20	23.50	23.80
48.0	4575	21.84	21.97	22.24	22.51	22.76	23.03	23.32	23.63	23.93
48.2	4583	21.96	22.09	22.36	22.63	22.88	23.16	23.45	23.76	24.06
48.4	4590	22.08	22.21	22.48	22.75	23.01	23.28	23.58	23.89	24.19
48.6	4598	22.20	22.33	22.60	22.87	23.13	23.41	23.71	24.02	24.32
48.8	4605	22.32	22.45	22.72	22.99	23.26	23.54	23.83	24.14	24.45

Alcohol table—Continued. 43.022

SCALE READING	INDEX OF REFRACTION	17.5° C	18° C	19° C	20° C	21° C	22° C	23° C	24° C	25° C
49.0	1.34613	22.44	22.57	22.84	23.12	23.38	23.66	23.96	24.27	24.59
49.2	4620	22.56	22.69	22.96	23.24	23.51	23.79	24.09	24.40	24.72
49.4	4628	22.68	22.81	23.08	23.36	23.63	23.92	24.22	24.53	24.85
49.6	4635	22.80	22.93	23.21	23.48	23.76	24.04	24.35	24.66	24.98
49.8	4643	22.92	23.05	23.33	23.61	23.88	24.17	24.48	24.79	25.11
50.0	4650	23.04	23.17	23.45	23.73	24.01	24.30	24.61	24.92	25.25
50.2	4658	23.16	23.30	23.57	23.85	24.13	24.43	24.74	25.05	25.38
50.4	4665	23.28	23.42	23.69	23.98	24.26	24.56	24.86	25.18	25.51
50.6	4672	23.40	23.54	23.81	24.10	24.38	24.69	24.99	25.32	25.65
50.8	4680	23.51	23.66	23.93	24.22	24.51	24.81	25.12	25.45	25.78
51.0	4687	23.63	23.78	24.05	24.35	24.64	24.94	25.25	25.58	25.91
51.2	4695	23.75	23.90	24.18	24.47	24.76	25.07	25.38	25.71	26.05
51.4	4702	23.87	24.02	24.30	24.59	24.89	25.20	25.51	25.84	26.18
51.6	4710	23.99	24.14	24.42	24.72	25.01	25.33	25.64	25.97	26.32
51.8	4717	24.11	24.26	24.54	24.84	25.14	25.46	25.77	26.11	26.45
52.0	4724	24.23	24.38	24.66	24.96	25.27	25.58	25.90	26.24	26.59
52.2	4732	24.36	24.50	24.79	25.09	25.39	25.71	26.03	26.37	26.72
52.4	4740	24.48	24.62	24.91	25.21	25.52	25.84	26.16	26.51	26.86
52.6	4747	24.60	24.74	25.03	25.34	25.65	25.97	26.29	26.64	26.99
52.8	4754	24.72	24.86	25.15	25.46	25.77	26.10	26.42	26.77	27.13
53.0	4762	24.84	24.98	25.28	25.59	25.90	26.23	26.56	26.91	27.27
53.2	4769	24.96	25.10	25.40	25.71	26.03	26.35	26.69	27.04	27.40
53.4	4777	25.08	25.23	25.52	25.84	26.15	26.48	26.82	27.17	27.54
53.6	4784	25.20	25.35	25.65	25.96	26.28	26.61	26.95	27.31	27.67
53.8	4792	25.32	25.47	25.77	26.09	26.41	26.74	27.08	27.44	27.81
54.0	4799	25.44	25.59	25.90	26.22	26.54	26.87	27.21	27.58	27.95
54.2	4806	25.56	25.71	26.02	26.34	26.67	27.00	27.35	27.71	28.08
54.4	4814	25.68	25.84	26.14	26.47	26.79	27.13	27.48	27.85	28.22
54.6	4821	25.81	25.96	26.27	26.59	26.92	27.26	27.61	27.98	28.36
54.8	4829	25.93	26.08	26.39	26.72	27.05	27.39	27.75	28.11	28.49
55.0	4836	26.05	26.20	26.52	26.85	27.18	27.52	27.88	28.25	28.63
55.2	4844	26.17	26.32	26.64	26.97	27.31	27.65	28.01	28.38	28.77
55.4	4851	26.29	26.45	26.76	27.10	27.43	27.78	28.15	28.52	28.90
55.6	4858	26.41	26.57	26.89	27.23	27.55	27.92	28.28	28.65	29.04
55.8	4866	26.53	26.69	27.01	27.35	27.69	28.05	28.41	28.78	29.18
56.0	4873	26.65	26.81	27.14	27.48	27.82	28.18	28.54	28.92	29.31
56.2	4880	26.78	26.93	27.26	27.60	27.94	28.31	28.68	29.05	29.45
56.4	4888	26.90	27.05	27.38	27.73	28.07	28.44	28.81	29.19	29.58
56.6	4895	27.02	27.18	27.51	27.85	28.20	28.56	28.94	29.32	29.72
56.8	4903	27.14	27.30	27.63	27.98	28.33	28.69	29.07	29.46	29.86
57.0	4910	27.26	27.42	27.75	28.10	28.46	28.82	29.20	29.59	29.99
57.2	4918	27.38	27.54	27.88	28.23	28.59	28.95	29.34	29.73	30.13
57.4	4925	27.50	27.66	28.00	28.35	28.72	29.08	29.47	29.86	30.27
57.6	4932	27.62	27.79	28.13	28.48	28.85	29.21	29.60	30.00	30.41
57.8	4940	27.75	27.91	28.25	28.60	28.97	29.34	29.73	30.14	30.55

43.022 *Alcohol table*—Continued.

SCALE READING	INDEX OF REFRACTION	17.5° C	18° C	19° C	20° C	21° C	22° C	23° C	24° C	25° C
58.0	1.34947	27.87	28.03	28.38	28.73	29.10	29.47	29.87	30.27	30.69
58.2	4954	27.99	28.15	28.50	28.86	29.23	29.60	29.99	30.41	30.83
58.4	4962	28.11	28.28	28.62	28.98	29.36	29.73	30.13	30.54	30.97
58.6	4969	28.23	28.40	28.75	29.11	29.48	29.86	30.26	30.68	31.11
58.8	4977	28.35	28.52	28.88	29.23	29.61	29.99	30.40	30.82	31.25
59.0	4984	28.47	28.64	29.00	29.36	29.74	30.13	30.53	30.95	31.40
59.2	4991	28.59	28.77	29.12	29.49	29.87	30.26	30.67	31.09	31.54
59.4	4999	28.71	28.89	29.25	29.61	29.99	30.39	30.81	31.23	31.68
59.6	5006	28.84	29.01	29.37	29.74	30.13	30.53	30.94	31.38	31.83
59.8	5014	28.96	29.13	29.50	29.87	30.26	30.66	31.08	31.52	31.97
60.0	5021	29.08	29.26	29.62	29.99	30.39	30.79	31.22	31.66	32.12
60.2	5028	29.20	29.38	29.74	30.12	30.52	30.93	31.36	31.80	32.27
60.4	5036	29.32	29.50	29.87	30.25	30.65	31.06	31.50	31.94	32.41
60.6	5043	29.45	29.63	29.99	30.38	30.78	31.20	31.64	32.09	32.56
60.8	5050	29.57	29.75	30.12	30.51	30.91	31.33	31.78	32.23	32.71
61.0	5058	29.69	29.87	30.25	30.64	31.05	31.47	31.92	32.38	32.86
61.2	5065	29.81	29.99	30.38	30.77	31.18	31.61	32.06	32.52	33.01
61.4	5073	29.93	30.12	30.50	30.90	31.32	31.74	32.20	32.67	33.16
61.6	5080	30.06	30.25	30.63	31.03	31.45	31.88	32.34	32.81	33.31
61.8	5087	30.18	30.37	30.76	31.16	31.59	32.01	32.49	32.96	33.46
62.0	5095	30.31	30.50	30.89	31.29	31.72	32.16	32.63	33.10	33.60
62.2	5102	30.43	30.63	31.01	31.43	31.86	32.30	32.77	33.25	33.75
62.4	5110	30.56	30.75	31.14	31.56	31.99	32.44	32.91	33.40	33.90
62.6	5117	30.69	30.88	31.28	31.69	32.13	32.58	33.06	33.55	34.05
62.8	5124	30.81	31.01	31.41	31.83	32.27	32.72	33.20	33.70	34.21
63.0	5132	30.94	31.14	31.54	31.96	32.41	32.87	33.35	33.84	34.36
63.2	5139	31.06	31.26	31.67	32.10	32.55	33.01	33.50	33.99	34.52
63.4	5146	31.19	31.39	31.80	32.23	32.69	33.15	33.64	34.15	34.67
63.6	5154	31.32	31.52	31.93	32.37	32.83	33.30	33.79	34.30	34.83
63.8	5161	31.45	31.65	32.07	32.51	32.97	33.44	33.93	34.45	34.98
64.0	5168	31.58	31.78	32.20	32.65	33.11	33.59	34.08	34.61	35.15
64.2	5176	31.70	31.91	32.34	32.79	33.25	33.73	34.23	34.76	35.31
64.4	5183	31.83	32.04	32.47	32.92	33.39	33.88	34.39	34.92	35.48
64.6	5190	31.96	32.17	32.60	33.06	33.53	34.02	34.54	35.07	35.64
64.8	5198	32.09	32.30	32.74	33.20	33.67	34.17	34.69	35.23	35.80
65.0	5205	32.22	32.43	32.87	33.34	33.82	34.32	34.84	35.39	35.97
65.2	5212	32.35	32.57	33.01	33.48	33.96	34.47	34.99	35.55	36.13
65.4	5220	32.48	32.70	33.15	33.62	34.10	34.61	35.15	35.71	36.30
65.6	5227	32.61	32.83	33.28	33.76	34.25	34.76	35.30	35.87	36.46
65.8	5234	32.75	32.96	33.42	33.90	34.40	34.91	35.46	36.02	36.63
66.0	5242	32.88	33.10	33.56	34.04	34.54	35.06	35.62	36.19	36.79
66.2	5249	33.01	33.23	33.70	34.18	34.69	35.22	35.77	36.35	36.96
66.4	5256	33.14	33.37	33.84	34.33	34.84	35.38	35.93	36.52	37.13
66.6	5264	33.28	33.51	33.98	34.47	34.99	35.53	36.09	36.68	37.30
66.8	5271	33.41	33.65	34.12	34.62	35.14	35.69	36.25	36.84	37.48

Alcohol table—Continued. 43.022

SCALE READING	INDEX OF REFRACTION	17.5° C	18° C	19° C	20° C	21° C	22° C	23° C	24° C	25° C
67.0	1.35278	33.55	33.79	34.26	34.76	35.29	35.84	36.41	37.01	37.65
67.2	5286	33.69	33.92	34.41	34.91	35.44	36.00	36.57	37.18	37.83
67.4	5293	33.82	34.06	34.55	35.05	35.60	36.16	36.73	37.35	38.00
67.6	5300	33.96	34.20	34.69	35.20	35.75	36.32	36.90	37.52	38.18
67.8	5308	34.09	34.34	34.84	35.35	35.90	36.48	37.06	37.69	38.35
68.0	5315	34.23	34.48	34.98	35.50	36.05	36.63	37.23	37.86	38.53
68.2	5322	34.36	34.62	35.13	35.65	36.21	36.79	37.39	38.03	38.70
68.4	5329	34.50	34.76	35.27	35.80	36.37	36.95	37.56	38.21	38.88
68.6	5337	34.64	34.90	35.42	35.95	36.52	37.12	37.73	38.38	39.06
68.8	5344	34.77	35.04	35.57	36.10	36.68	37.28	37.90	38.56	39.24
69.0	5351	34.91	35.19	35.71	36.25	36.84	37.45	38.07	38.73	39.43
69.2	5359	35.04	35.33	35.86	36.41	36.99	37.61	38.24	38.90	39.61
69.4	5366	35.19	35.47	36.01	36.56	37.15	37.78	38.41	39.08	39.80
69.6	5373	35.34	35.62	36.16	36.72	37.32	37.94	38.58	39.26	39.98
69.8	5381	35.49	35.76	36.31	36.87	37.48	38.11	38.75	39.45	40.17
70.0	5388	35.64	35.91	36.46	37.02	37.64	38.28	38.92	39.63	40.35
70.2	5395	35.78	36.05	36.61	37.19	37.80	38.45	39.10	39.81	40.53
70.4	5402	35.93	36.20	36.76	37.35	37.97	38.61	39.28	39.99	40.72
70.6	5410	36.08	36.35	36.92	37.51	38.13	38.78	39.46	40.17	40.90
70.8	5417	36.23	36.50	37.07	37.67	38.30	38.95	39.64	40.35	41.08
71.0	5424	36.38	36.65	37.23	37.83	38.47	39.12	39.82	40.54	41.27
71.2	5432	36.53	36.80	37.39	37.99	38.63	39.30	40.00	40.72	40.46
71.4	5439	36.68	36.95	37.55	38.16	38.80	39.48	40.18	40.90	41.64
71.6	5446	36.83	37.11	37.71	38.32	38.97	39.65	40.36	41.08	41.83
71.8	5454	36.98	37.27	37.87	38.49	39.14	39.83	40.54	41.27	42.02
72.0	5461	37.13	37.42	38.02	38.65	39.31	40.01	40.72	41.45	42.21
72.2	5468	37.29	37.58	38.19	38.82	39.49	40.18	40.90	41.64	42.40
72.4	5475	37.44	37.73	38.35	38.98	39.66	40.36	41.08	41.82	42.58
72.6	5483	37.60	37.89	38.51	39.16	39.83	40.54	41.26	42.01	42.77
72.8	5490	37.75	38.05	38.67	39.33	40.01	40.71	41.45	42.19	42.96
73.0	5497	37.91	38.21	38.84	39.50	40.18	40.88	41.63	42.38	43.15
73.2	5504	38.06	38.37	39.00	39.67	40.36	41.06	41.81	42.56	43.33
73.4	5512	38.22	38.53	39.17	39.84	40.53	41.24	41.99	42.75	43.52
73.6	5519	38.38	38.69	39.34	40.02	40.70	41.42	42.17	42.93	43.70
73.8	5526	38.54	38.85	39.50	40.19	40.88	41.60	42.36	43.12	43.89
74.0	5533	38.70	39.01	39.67	40.36	41.05	41.78	42.54	43.31	44.08
74.2	5541	38.86	39.18	39.84	40.53	41.23	41.96	42.72	43.49	44.28
74.4	5548	39.02	39.34	40.01	40.71	41.41	42.15	42.91	43.68	44.48
74.6	5555	39.18	39.51	40.18	40.88	41.59	42.33	43.09	43.86	44.67
74.8	5563	39.35	39.68	40.35	41.05	41.77	42.51	43.28	44.05	44.87
75.0	5570	39.51	39.84	40.53	41.23	41.95	42.70	43.46	44.25	45.07
75.2	5577	39.68	40.01	40.70	41.41	42.13	42.88	43.65	44.44	45.29
75.4	5584	39.84	40.18	40.87	41.58	42.31	43.07	43.83	44.63	45.50
75.6	5592	40.01	40.35	41.04	41.76	42.49	43.25	44.02	44.83	45.71
75.8	5599	40.18	40.53	41.22	41.94	42.67	43.44	44.21	45.03	45.92

43.022 *Alcohol table*—Concluded.

SCALE READING	INDEX OF REFRACTION	17.5° C	18° C	19° C	20° C	21° C	22° C	23° C	24° C	25° C
76.0	1.35606	40.35	40.70	41.40	42.12	42.85	43.63	44.41	45.24	46.12
76.2	5613	40.53	40.87	41.57	42.30	43.04	43.81	44.60	45.44	46.34
76.4	5621	40.70	41.04	41.75	42.48	43.22	44.00	44.80	45.65	46.56
76.6	5628	40.87	41.22	41.92	42.66	43.41	44.19	44.99	45.86	46.78
76.8	5635	41.04	41.39	42.10	42.84	43.60	44.38	45.19	46.07	47.00
77.0	5642	41.22	41.57	42.28	43.02	43.79	44.57	45.40	46.29	47.23
77.2	5650	41.39	41.74	42.46	43.20	43.97	44.76	45.60	46.51	47.45
77.4	5657	41.57	41.91	42.63	43.39	44.16	44.95	45.81	46.73	47.68
77.6	5664	41.75	42.09	42.81	43.57	44.35	45.15	46.01	46.95	47.91
77.8	5671	41.92	42.26	42.99	43.76	44.54	45.35	46.23	47.17	48.14
78.0	5678	42.09	42.43	43.17	43.94	44.73	45.56	46.45	47.40	48.37
78.2	5686	42.26	42.61	43.36	44.13	44.92	45.76	46.67	47.63	48.60
78.4	5693	42.44	42.78	43.54	44.32	45.12	45.96	46.89	47.85	48.84
78.6	5700	42.61	42.96	43.72	44.51	45.32	46.17	47.11	48.08	49.07
78.8	5707	42.78	43.14	43.91	44.70	45.52	46.39	47.34	48.31	49.31
79.0	5715	42.95	43.32	44.09	44.89	45.72	46.61	47.56	48.53	49.54
79.2	5722	43.13	43.50	44.28	45.08	45.92	46.83	47.79	48.76	49.77
79.4	5729	43.31	43.68	44.47	45.28	46.13	47.04	48.01	48.99	50.01
79.6	5736	43.49	43.86	44.65	45.48	46.34	47.26	48.23	49.22	50.24
79.8	5744	43.67	44.05	44.84	45.68	46.56	47.48	48.46	49.45	50.48
80.0	5751	43.85	44.24	45.04	45.88	46.77	47.70	48.68	49.68	50.71

Percentages by wt corresponding to various percentages by vol. at 15.56°C (60°F) in mixts of ethyl alcohol and H_2O[a] **43.023**

PER CENT ALCOHOL BY VOLUME AT 60° F.	PER CENT ALCOHOL BY WEIGHT	DIFFERENCES	PER CENT ALCOHOL BY VOLUME AT 60° F.	PER CENT ALCOHOL BY WEIGHT	DIFFERENCES
0	0.000	....	50	42.487	....
1	0.795	0.795	51	43.428	0.941
2	1.593	.798	52	44.374	.946
3	2.392	.799	53	45.326	.952
4	3.194	.802	54	46.283	.957
		.804			.962
5	3.998		55	47.245	
6	4.804	.806	56	48.214	.969
7	5.612	.808	57	49.187	.973
8	6.422	.810	58	50.167	.980
9	7.234	.812	59	51.154	.987
		.813			.993
10	8.047		60	52.147	
11	8.862	.815	61	53.146	.999
12	9.679	.817	62	54.152	1.006
13	10.497	.818	63	55.165	1.013
14	11.317	.820	64	56.184	1.019
		.821			1.024
15	12.138		65	57.208	
16	12.961	.823	66	58.241	1.033
17	13.786	.825	67	59.279	1.038
18	14.612	.826	68	60.325	1.046
19	15.440	.828	69	61.379	1.054
		.829			1.062
20	16.269		70	62.441	
21	17.100	.831	71	63.511	1.070
22	17.933	.833	72	64.588	1.077
23	18.768	.835	73	65.674	1.086
24	19.604	.836	74	66.768	1.094
		.839			1.102
25	20.443		75	67.870	
26	21.285	.842	76	68.982	1.112
27	22.127	.842	77	70.102	1.120
28	22.973	.846	78	71.234	1.132
29	23.820	.847	79	72.375	1.141
		.850			1.151
30	24.670		80	73.526	
31	25.524	.854	81	74.686	1.160
32	26.382	.858	82	75.858	1.172
33	27.242	.860	83	77.039	1.181
34	28.104	.862	84	78.233	1.194
		.867			1.208
35	28.971		85	79.441	
36	29.842	.871	86	80.662	1.221
37	30.717	.875	87	81.897	1.235
38	31.596	.879	88	83.144	1.247
39	32.478	.882	89	84.408	1.264
		.886			1.281
40	33.364		90	85.689	
41	34.254	.890	91	86.989	1.300
42	35.150	.896	92	88.310	1.321
43	36.050	.900	93	89.652	1.342
44	36.955	.905	94	91.025	1.373
		.910			1.398
45	37.865		95	92.423	
46	38.778	.913	96	93.851	1.428
47	39.697	.919	97	95.315	1.464
48	40.622	.925	98	96.820	1.505
49	41.551	.929	99	98.381	1.561
		.936			1.619
50	42.487	....	100	100.000	....

[a] *Bureau of Standards Circular* No. 19, p. 18 (1924).

43.024 *Correction table for specific gravity of milk (Quévenne lactometer)*[a]

LACTOMETER	TEMPERATURE (F)																				LACTOMETER
	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	
20	19.3	19.4	19.4	19.5	19.6	19.7	19.8	19.9	19.9	20.0	20.1	20.2	20.2	20.3	20.4	20.5	20.6	20.7	20.9	21.0	20
21	20.3	20.3	20.4	20.5	20.6	20.7	20.8	20.9	20.9	21.0	21.1	21.2	21.3	21.4	21.5	21.6	21.7	21.8	22.0	22.1	21
22	21.3	21.3	21.4	21.5	21.6	21.7	21.8	21.9	21.9	22.0	22.1	22.2	22.3	22.4	22.5	22.6	22.7	22.8	23.0	23.1	22
23	22.3	22.3	22.4	22.5	22.6	22.7	22.8	22.8	22.9	23.0	23.1	23.2	23.3	23.4	23.5	23.6	23.7	23.8	24.0	24.1	23
24	23.3	23.3	23.4	23.5	23.6	23.6	23.7	23.8	23.9	24.0	24.1	24.2	24.3	24.4	24.5	24.6	24.7	24.9	25.0	25.1	24
25	24.2	24.3	24.4	24.5	24.6	24.6	24.7	24.8	24.9	25.0	25.1	25.2	25.3	25.4	25.5	25.6	25.7	25.9	26.0	26.1	25
26	25.2	25.2	25.3	25.4	25.5	25.6	25.7	25.8	25.9	26.0	26.1	26.2	26.3	26.5	26.6	26.7	26.8	27.0	27.1	27.2	26
27	26.2	26.2	26.3	26.4	26.5	26.6	26.7	26.8	26.9	27.0	27.1	27.3	27.4	27.5	27.6	27.7	27.8	28.0	28.1	28.2	27
28	27.1	27.2	27.3	27.4	27.5	27.6	27.7	27.8	27.9	28.0	28.1	28.3	28.4	28.5	28.6	28.7	28.8	29.0	29.1	29.2	28
29	28.1	28.2	28.3	28.4	28.5	28.6	28.7	28.8	28.9	29.0	29.1	29.3	29.4	29.5	29.6	29.7	29.9	30.1	30.2	30.3	29
30	29.1	29.1	29.2	29.3	29.4	29.6	29.7	29.8	29.9	30.0	30.1	30.3	30.4	30.5	30.7	30.8	30.9	31.1	31.2	31.3	30
31	30.0	30.1	30.2	30.3	30.4	30.5	30.6	30.8	30.9	31.0	31.2	31.3	31.4	31.5	31.7	31.8	31.9	32.1	32.2	32.4	31
32	31.0	31.1	31.2	31.3	31.4	31.5	31.6	31.7	31.9	32.0	32.2	32.3	32.5	32.6	32.7	32.9	33.0	33.2	33.3	33.4	32
33	31.9	32.0	32.1	32.3	32.4	32.5	32.6	32.7	32.9	33.0	33.2	33.3	33.5	33.6	33.8	33.9	34.0	34.2	34.3	34.5	33
34	32.9	33.0	33.1	33.2	33.3	33.5	33.6	33.7	33.9	34.0	34.2	34.3	34.5	34.6	34.8	34.9	35.0	35.2	35.3	35.5	34
35	33.8	33.9	34.0	34.2	34.3	34.5	34.6	34.7	34.9	35.0	35.2	35.3	35.5	35.6	35.8	36.9	36.1	36.2	36.4	36.5	35

[a] Paul G. Heineman, "Milk," p. 144. W. B. Saunders Co. (1921).

Table for detg solids in milk from any given specific gravity and percentage of fat (Shaw and Eckles) **43.025**

(Results expressed as total solids, per cent)

PERCENTAGE OF FAT	LACTOMETER READING AT 60° F. (QUEVENNE DEGREES).										
	26	27	28	29	30	31	32	33	34	35	36
2.00	8.90	9.15	9.40	9.65	9.90	10.15	10.40	10.66	10.91	11.16	11.41
2.05	8.96	9.21	9.46	9.71	9.96	10.21	10.46	10.72	10.97	11.22	11.47
2.10	9.02	9.27	9.52	9.77	10.02	10.27	10.52	10.78	11.03	11.28	11.53
2.15	9.08	9.33	9.58	9.83	10.08	10.33	10.58	10.84	11.09	11.34	11.59
2.20	9.14	9.39	9.64	9.89	10.14	10.39	10.64	10.90	11.15	11.40	11.65
2.25	9.20	9.45	9.70	9.95	10.20	10.45	10.70	10.96	11.21	11.46	11.71
2.30	9.26	9.51	9.76	10.01	10.26	10.51	10.76	11.02	11.27	11.52	11.77
2.35	9.32	9.57	9.82	10.07	10.32	10.57	10.82	11.08	11.33	11.58	11.83
2.40	9.38	9.63	9.88	10.13	10.38	10.63	10.88	11.14	11.39	11.64	11.89
2.45	9.44	9.69	9.94	10.19	10.44	10.69	10.94	11.20	11.45	11.70	11.95
2.50	9.50	9.75	10.00	10.25	10.50	10.75	11.00	11.26	11.51	11.76	12.01
2.55	9.56	9.81	10.06	10.31	10.56	10.81	11.06	11.32	11.57	11.82	12.07
2.60	9.62	9.87	10.12	10.37	10.62	10.87	11.12	11.38	11.63	11.88	12.13
2.65	9.68	9.93	10.18	10.43	10.68	10.93	11.18	11.44	11.69	11.94	12.19
2.70	9.74	9.99	10.24	10.49	10.74	10.99	11.24	11.50	11.75	12.00	12.25
2.75	9.80	10.05	10.30	10.55	10.80	11.05	11.31	11.56	11.81	12.06	12.31
2.80	9.86	10.11	10.36	10.61	10.86	11.11	11.37	11.62	11.87	12.12	12.37
2.85	9.92	10.17	10.42	10.67	10.92	11.17	11.43	11.68	11.93	12.18	12.43
2.90	9.98	10.23	10.48	10.73	10.98	11.23	11.49	11.74	11.99	12.24	12.49
2.95	10.04	10.29	10.54	10.79	11.04	11.30	11.55	11.80	12.05	12.30	12.55
3.00	10.10	10.35	10.60	10.85	11.10	11.36	11.61	11.86	12.11	12.36	12.61
3.05	10.16	10.41	10.66	10.91	11.17	11.42	11.67	11.92	12.17	12.42	12.68
3.10	10.22	10.47	10.72	10.97	11.23	11.48	11.73	11.98	12.23	12.48	12.74
3.15	10.28	10.53	10.78	11.03	11.29	11.54	11.79	12.04	12.29	12.55	12.80
3.20	10.34	10.59	10.84	11.09	11.35	11.60	11.85	12.10	12.35	12.61	12.86
3.25	10.40	10.65	10.90	11.16	11.41	11.66	11.91	12.16	12.42	12.67	12.92
3.30	10.46	10.71	10.96	11.22	11.47	11.72	11.97	12.22	12.48	12.73	12.98
3.35	10.52	10.77	11.03	11.28	11.53	11.78	12.03	12.28	12.54	12.79	13.04
3.40	10.58	10.83	11.09	11.34	11.59	11.84	12.09	12.34	12.60	12.85	13.10
3.45	10.64	10.89	11.15	11.40	11.65	11.90	12.15	12.40	12.66	12.91	13.16
3.50	10.70	10.95	11.21	11.46	11.71	11.96	12.21	12.46	12.72	12.97	13.22
3.55	10.76	11.02	11.27	11.52	11.77	12.02	12.27	12.52	12.78	13.03	13.28
3.60	10.82	11.08	11.33	11.58	11.83	12.08	12.33	12.58	12.84	13.09	13.34
3.65	10.88	11.14	11.39	11.64	11.89	12.14	12.39	12.64	12.90	13.15	13.40
3.70	10.94	11.20	11.45	11.70	11.95	12.20	12.45	12.70	12.96	13.21	13.46
3.75	11.00	11.26	11.51	11.76	12.01	12.26	12.51	12.76	13.02	13.27	13.52
3.80	11.06	11.32	11.57	11.82	12.07	12.32	12.57	12.82	13.08	13.33	13.58
3.85	11.12	11.38	11.63	11.88	12.13	12.38	12.63	12.88	13.14	13.39	13.64
3.90	11.18	11.44	11.69	11.94	12.19	12.44	12.69	12.94	13.20	13.45	13.70
3.95	11.24	11.50	11.75	12.00	12.25	12.50	12.75	13.00	13.26	13.51	13.77
4.00	11.30	11.56	11.81	12.06	12.31	12.56	12.81	13.06	13.32	13.57	13.83
4.05	11.36	11.62	11.87	12.12	12.37	12.62	12.87	13.12	13.38	13.63	13.89
4.10	11.42	11.68	11.93	12.18	12.43	12.68	12.93	13.18	13.44	13.69	13.95
4.15	11.48	11.74	11.99	12.24	12.49	12.74	12.99	13.25	13.50	13.76	14.01
4.20	11.54	11.80	12.05	12.30	12.55	12.80	13.05	13.31	13.56	13.82	14.07
4.25	11.60	11.86	12.11	12.36	12.61	12.86	13.12	13.37	13.62	13.88	14.13
4.30	11.66	11.92	12.17	12.42	12.67	12.92	13.18	13.43	13.68	13.94	14.19
4.35	11.72	11.98	12.23	12.48	12.73	12.98	13.24	13.49	13.74	14.00	14.25
4.40	11.78	12.04	12.29	12.54	12.79	13.04	13.30	13.55	13.80	14.06	14.31
4.45	11.84	12.10	12.35	12.60	12.85	13.10	13.36	13.61	13.86	14.12	14.37
4.50	11.90	12.16	12.41	12.66	12.91	13.16	13.42	13.67	13.92	14.18	14.43
4.55	11.97	12.22	12.47	12.72	12.97	13.22	13.48	13.73	13.98	14.24	14.49
4.60	12.03	12.28	12.53	12.78	13.03	13.28	13.54	13.79	14.04	14.30	14.55
4.65	12.09	12.34	12.59	12.84	13.09	13.34	13.60	13.85	14.10	14.36	14.61
4.70	12.15	12.40	12.65	12.90	13.15	13.40	13.66	13.91	14.16	14.42	14.67
4.75	12.21	12.46	12.71	12.96	13.21	13.46	13.72	13.97	14.22	14.48	14.73
4.80	12.27	12.52	12.77	13.02	13.27	13.52	13.78	14.03	14.28	14.54	14.79
4.85	12.33	12.58	12.83	13.08	13.33	13.58	13.84	14.09	14.34	14.60	14.85
4.90	12.39	12.64	12.89	13.14	13.39	13.64	13.90	14.15	14.40	14.66	14.91
4.95	12.45	12.70	12.95	13.20	13.45	13.70	13.96	14.21	14.46	14.72	14.97
5.00	12.51	12.76	13.01	13.26	13.51	13.76	14.02	14.27	14.52	14.78	15.03
5.05	12.57	12.82	13.07	13.32	13.57	13.83	14.08	14.33	14.58	14.84	15.09
5.10	12.63	12.88	13.13	13.38	13.63	13.89	14.14	14.39	14.64	14.90	15.15
5.15	12.69	12.94	13.19	13.44	13.69	13.95	14.20	14.45	14.70	14.96	15.21
5.20	12.75	13.00	13.25	13.50	13.75	14.01	14.26	14.51	14.76	15.02	15.27
5.25	12.81	13.06	13.31	13.56	13.81	14.07	14.32	14.57	14.82	15.08	15.33
5.30	12.87	13.12	13.37	13.62	13.87	14.13	14.38	14.63	14.88	15.14	15.39
5.35	12.93	13.18	13.43	13.68	13.93	14.19	14.44	14.70	14.95	15.20	15.45
5.40	12.99	13.24	13.49	13.74	14.00	14.25	14.50	14.76	15.01	15.26	15.51
5.45	13.05	13.30	13.55	13.80	14.06	14.31	14.56	14.82	15.07	15.32	15.57

43.025 *Table for detg total solids in milk from any given specific gravity and percentage of fat (Shaw and Eckles)*—Concluded.

PER-CENTAGE OF FAT	LACTOMETER READING AT 60° F. (QUEVENNE DEGREES)										
	26	27	28	29	30	31	32	33	34	35	36
5.50	13.11	13.36	13.61	13.86	14.12	14.37	14.62	14.88	15.13	15.38	15.63
5.55	13.17	13.42	13.67	13.93	14.18	14.43	14.69	14.94	15.19	15.44	15.69
5.60	13.23	13.48	13.73	13.99	14.24	14.49	14.75	15.00	15.25	15.50	15.75
5.65	13.29	13.54	13.79	14.05	14.30	14.55	14.81	15.06	15.31	15.56	15.81
5.70	13.35	13.60	13.85	14.11	14.36	14.61	14.87	15.12	15.37	15.62	15.87
5.75	13.41	13.66	13.91	14.17	14.42	14.68	14.93	15.18	15.43	15.68	15.93
5.80	13.47	13.72	13.97	14.23	14.48	14.74	14.99	15.24	15.49	15.74	15.99
5.85	13.53	13.78	14.04	14.29	14.54	14.80	15.05	15.30	15.55	15.80	16.06
5.90	13.59	13.84	14.10	14.35	14.60	14.86	15.11	15.36	15.61	15.86	16.12
5.95	13.65	13.90	14.16	14.41	14.66	14.92	15.17	15.42	15.67	15.92	16.18
6.00	13.71	13.96	14.22	14.47	14.72	14.98	15.23	15.48	15.73	15.98	16.24
6.05	13.77	14.02	14.28	14.53	14.78	15.04	15.29	15.54	15.79	16.04	16.30
6.10	13.83	14.08	14.34	14.59	14.84	15.10	15.35	15.60	15.85	16.10	16.35
6.15	13.89	14.14	14.40	14.65	14.90	15.16	15.41	15.66	15.91	16.16	16.42
6.20	13.95	14.20	14.46	14.71	14.96	15.22	15.47	15.72	15.97	16.22	16.48
6.25	14.01	14.26	14.52	14.77	15.02	15.28	15.53	15.78	16.03	16.28	16.54
6.30	14.07	14.32	14.58	14.83	15.08	15.34	15.59	15.84	16.09	16.34	16.60
6.35	14.13	14.38	14.64	14.90	15.14	15.40	15.65	15.90	16.15	16.40	16.66
6.40	14.19	14.44	14.70	14.96	15.20	15.46	15.71	15.96	16.21	16.46	16.72
6.45	14.25	14.50	14.76	15.02	15.26	15.52	15.77	16.02	16.27	16.52	16.78
6.50	14.31	14.56	14.82	15.08	15.32	15.58	15.83	16.08	16.33	16.58	16.84
6.55	14.37	14.62	14.88	15.14	15.38	15.64	15.89	16.14	16.39	16.64	16.90
6.60	14.43	14.68	14.94	15.20	15.44	15.70	15.95	16.20	16.45	16.70	16.96
6.65	14.49	14.74	15.00	15.26	15.50	15.76	16.01	16.26	16.51	16.76	17.02
6.70	14.55	14.80	15.06	15.32	15.56	15.82	16.07	16.32	16.57	16.82	17.08
6.75	14.61	14.86	15.12	15.38	15.62	15.88	16.13	16.38	16.63	16.88	17.14
6.80	14.67	14.92	15.18	15.44	15.68	15.94	16.19	16.44	16.69	16.94	17.20
6.85	14.73	14.98	15.24	15.50	15.74	16.00	16.25	16.50	16.75	17.00	17.26
6.90	14.79	15.04	15.30	15.56	15.80	16.06	16.31	16.56	16.81	17.06	17.32
6.95	14.85	15.10	15.36	15.62	15.86	16.12	16.37	16.62	16.87	17.12	17.38

Proportional Parts

LACTOMETER FRACTION	FRACTION TO BE ADDED TO TOTAL SOLIDS	LACTOMETER FRACTION	FRACTION TO BE ADDED TO TOTAL SOLIDS	LACTOMETER FRACTION	FRACTION TO BE ADDED TO TOTAL SOLIDS
0.1	0.03	0.4	0.10	0.7	0.18
.2	.05	.5	.13	.8	.20
.3	.08	.6	.15	.9	.23

Table giving Proportional Parts shows amount to be added when lactometer readings are in whole numbers and decimals.

Density of carbon dioxide (Parr)[a] 43.026

(Wt in mg of 1 ml of CO_2 at 700–770 mm pressure and 10–30°C. Corrected for aq. vapor and barometer readings on glass scale. Calcd from 1.976 equals wt of liter of CO_2 at 0°C, 760 mm pressure, and 41° latitude.)

mm.	10°	11°	12°	13°	14°	15°	16°	17°	18°	19°
700	1.7288	1.7201	1.7113	1.7020	1.6927	1.6863	1.6799	1.6716	1.6632	1.6547
702	.7338	.7252	.7164	.7072	.6980	.6914	.6848	.6765	.6680	.6595
704	.7388	.7302	.7215	.7124	.7033	.6965	.6897	.6813	.6729	.6644
706	.7438	.7353	.7266	.7176	.7086	.7016	.6946	.6862	.6778	.6692
708	.7488	.7403	.7317	.7228	.7139	.7067	.6995	.6911	.6826	.6741
710	.7538	.7453	.7368	.7280	.7192	.7118	.7044	.6960	.6874	.6789
712	.7588	.7504	.7419	.7332	.7245	.7169	.7092	.7008	.6922	.6837
714	.7638	.7555	.7470	.7384	.7298	.7220	.7141	.7057	.6970	.6886
716	.7688	.7605	.7521	.7436	.7351	.7271	.7190	.7106	.7019	.6934
718	.7738	.7656	.7572	.7488	.7404	.7322	.7239	.7154	.7068	.6983
720	.7788	.7706	.7623	.7540	.7457	.7373	.7288	.7203	.7117	.7031
722	.7838	.7756	.7673	.7590	.7506	.7422	.7337	.7252	.7166	.7079
724	.7888	.7806	.7723	.7639	.7555	.7471	.7386	.7301	.7215	.7128
726	.7938	.7856	.7773	.7689	.7605	.7520	.7435	.7349	.7263	.7176
728	.7988	.7905	.7822	.7738	.7654	.7569	.7484	.7398	.7312	.7225
730	.8038	.7955	.7872	.7788	.7703	.7618	.7533	.7447	.7360	.7273
732	.8089	.8005	.7921	.7837	.7752	.7667	.7582	.7496	.7409	.7321
734	.8139	.8055	.7971	.7887	.7802	.7717	.7631	7545	.7458	.7370
736	.8189	.8105	.8021	.7936	.7851	.7766	.7680	.7593	.7506	.7418
738	.8239	.8155	.8071	.7986	.7901	.7815	.7729	.7642	.7555	.7467
740	.8288	.8204	.8120	.8035	.7950	.7864	.7778	.7691	7603	.7515
742	.8338	.8254	.8170	.8085	.7999	.7913	.7827	.7740	.7652	.7564
744	.8388	.8304	.8219	.8134	.8048	.7962	.7875	.7788	.7700	.7612
746	.8439	.8354	.8269	.8184	.8098	.8011	.7924	.7837	.7749	.7661
748	.8489	.8404	.8319	.8233	.8147	.8060	.7973	.7886	.7798	.7709
750	.8539	.8454	.8368	.8282	.8196	.8109	.8022	.7934	.7846	.7757
752	.8589	.8504	.8418	.8332	.8246	.8159	.8072	.7984	.7895	.7806
754	.8639	.8554	.8468	.8382	.8295	.8208	.8120	.8032	.7944	.7854
756	.8689	.8603	.8517	.8431	.8344	.8257	.8169	.8081	.7992	.7902
758	.8739	.8653	.8567	.8481	.8394	.8306	.8218	.8130	.8041	.7951
760	.8789	.8703	.8617	.8530	.8443	.8355	.8267	.8178	.8089	.7999
762	.8839	.8753	.8667	.8580	.8492	.8404	.8316	.8227	.8138	.8048
764	.8890	.8803	.8716	.8629	.8541	.8453	.8365	.8276	.8187	.8096
766	.8940	.8853	.8766	.8679	.8591	.8503	.8414	.8325	.8235	.8144
768	.8990	.8903	.8816	.8728	.8640	.8552	.8463	.8374	.8284	.8193
770	.9040	.8953	.8865	.8777	.8689	.8601	.8512	.8422	.8332	.8241

[a] *J. Am. Chem. Soc.*, **31**, 237 (1909). Values of 700–718 mm were calcd by formula given by Parr.

43.026 *Density of carbon dioxide (Parr)*—Concluded.

20°	21°	22°	23°	24°	25°	26°	27°	28°	29°	30°	mm.
1.6462	1.6370	1.6278	1.6195	1.6112	1.6021	1.5930	1.5837	1.5744	1.5649	1.5554	700
.6510	.6419	.6327	.6243	.6160	.6068	.5977	.5884	.5791	.5696	.5600	702
.6558	.6467	.6376	.6292	.6207	.6116	.6025	.5931	.5838	.5742	.5647	704
.6607	.6516	.6425	.6340	.6254	.6163	.6072	.5979	.5885	.5789	.5693	706
.6655	.6564	.6474	.6388	.6302	.6211	.6119	.6026	.5932	.5836	.5740	708
.6703	.6613	.6522	.6436	.6350	.6258	.6166	.6073	.5978	.5882	.5786	710
.6751	.6662	.6571	.6485	.6397	.6305	.6214	.6120	.6025	.5929	.5832	712
.6799	.6710	.6620	.6533	.6444	.6353	.6261	.6167	.6072	.5976	.5879	714
.6848	.6759	.6670	.6581	.6492	.6400	.6308	.6215	.6119	.6023	.5925	716
.6896	.6807	.6718	.6629	.6540	.6448	.6356	.6262	.6166	.6069	.5972	718
.6944	.6856	.6767	.6678	.6587	.6495	.6403	.6309	.6213	.6116	.6018	720
.6992	.6904	.6815	.6726	.6635	.6543	.6450	.6356	.6260	.6163	.6065	722
.7041	.6953	.6863	.6773	.6682	.6590	.6497	.6403	.6307	.6210	.6111	724
.7089	.7001	.6911	.6821	.6730	.6638	.6544	.6450	.6354	.6256	.6157	726
.7137	.7049	.6959	.6869	.6778	.6685	.6591	.6497	.6401	.6303	.6204	728
.7185	.7097	.7007	.6917	.6825	.6732	.6638	.5544	.6448	.6350	.6251	730
.7233	.7145	.7055	.6964	.6872	.6779	.6685	.6591	.6494	.6396	.6297	732
.7282	.7193	.7103	.7012	.6920	.6827	.6733	.6638	.6541	.6443	.6343	734
.7330	.7241	.7151	.7060	.6968	.6875	.6780	.6685	.6588	.6490	.6390	736
.7378	.7289	.7199	.7107	.7015	.6922	.6827	.6732	.6635	.6537	.6437	738
.7426	.7337	.7247	.7155	.7063	.6969	.6874	.6778	.6681	.6583	.6483	740
.7475	.7385	.7295	.7203	.7111	.7017	.6922	.6826	.6729	.6630	.6530	742
.7523	.7433	.7342	.7250	.7158	.7064	.6969	.6873	.6776	.6677	.6577	744
.7571	.7481	.7390	.7298	.7206	.7112	.7016	.6920	.6822	.6723	.6623	746
.7619	.7529	.7438	.7346	.7253	.7159	.7063	.6967	.6869	.6770	.6670	748
.7667	.7577	.7486	.7394	.7301	.7206	.7110	.7014	.6916	.6817	.6716	750
.7716	.7625	.7534	.7441	.7348	.7254	.7158	.7061	.6963	.6864	.6763	752
.7764	.7673	.7582	.7489	.7396	.7301	.7205	.7108	.7010	.6910	.6809	754
.7812	.7721	.7630	.7537	.7443	.7348	.7252	.7155	.7057	.6957	.6856	756
.7861	.7770	.7678	.7585	.7491	.7396	.7300	.7202	.7104	.7004	.6903	758
.7909	.7818	.7725	.7632	.7538	.7443	.7347	.7249	.7150	.7050	.6949	760
.7957	.7866	.7773	.7680	.7586	.7490	.7394	.7296	.7197	.7097	.6996	762
.8005	.7914	.7821	.7728	.7633	.7538	.7441	.7343	.7244	.7144	.7042	764
.8053	.7962	.7869	.7776	.7681	.7585	.7488	.7390	.7291	.7191	.7089	766
.8102	.8010	.7917	.7823	.7728	.7633	.7535	.7437	.7338	.7237	.7135	768
.8150	.8058	.7965	.7871	.7776	.7680	.7582	.7484	.7385	.7284	.7182	770

Correction factors for gasometric determination of carbon dioxide[a] 43.027
(Based on sample weighing 1.7000 g)

(Multiply number of ml of gas evolved from 1.7000 g of sample by factor that corresponds with existing atmospheric conditions and divide by 10 to obtain % CO_2 by wt in sample.)

°C.	15.0°	15.5°	16.0°	16.5°	17.0°	17.5°	18.0°	18.5°	
mm.									*inches*
700	0.99194	0.99006	0.98818	0.98573	0.98329	0.98082	0.97835	0.97585	27.56
702	0.99494	0.99300	0.99106	0.98862	0.98618	0.98368	0.98118	0.97868	27.64
704	0.99794	0.99544	0.99394	0.99147	0.98900	0.98653	0.98406	0.98156	27.72
706	1.00094	0.99886	0.99682	0.99435	0.99188	0.98941	0.98694	0.98406	27.80
708	1.00394	1.00183	0.99971	0.99723	0.99476	0.99226	0.98976	0.98726	27.87
710	1.00694	1.00477	1.00259	1.00012	0.99765	0.99512	0.99259	0.99009	27.95
712	1.00994	1.00767	1.00541	1.00294	1.00047	0.99795	0.99541	0.99291	28.03
714	1.01294	1.01061	1.00829	1.00582	1.00335	1.00080	1.99824	0.99576	28.11
716	1.01594	1.01356	1.01118	1.00871	1.00624	1.00368	1.00112	0.99861	28.19
718	1.01894	1.01650	1.01406	1.01156	1.00906	1.00653	1.00400	1.00150	28.27
720	1.02194	1.01949	1.01694	1 01444	1.01194	1.00941	1.00688	1.00435	28.35
722	1.02482	1.02232	1.01982	1.01732	1.01482	1.01229	1.00976	1.00720	28.43
724	1.02771	1.02521	1.02271	1.02021	1.01771	1.01518	1.01265	1.01009	28.50
726	1.03059	1.02809	1.02559	1.02306	1.02053	1.01800	1.01574	1.01291	28.58
728	1.03347	1.03097	1.02847	1.02594	1.02341	1.02088	1.01835	1.01580	28.66
730	1.03635	1.03385	1.03135	1.02882	1.02629	1.02374	1.02118	1.01862	28.74
732	1.03924	1.03674	1.03424	1.03171	1.02918	1.02662	1.02406	1.02147	28.82
734	1.04218	1.03915	1.03712	1.03459	1.03206	1.02950	1.02694	1.02435	28.90
736	1.04506	1.04253	1.04000	1.03744	1.03488	1.03232	1.02976	1.02718	28.98
738	1.04794	1.04541	1.04288	1.04037	1.03776	1.03521	1.03265	1.03006	29.06
740	1.05082	1.04829	1.04576	1.04321	1.04065	1.03806	1.03547	1.03288	29.13
742	1.05371	1.05118	1.04865	1.04609	1.04353	1.04094	1.03835	1.03577	29.21
744	1.05659	1.05403	1.05147	1.04991	1.04635	1.04377	1.04118	1.03859	29.29
746	1.05947	1.05691	1.05435	1.05180	1.04924	1.04665	1.04406	1.04147	29.37
748	1.06235	1.05929	1.05724	1.05418	1.05212	1.04953	1.04694	1.04433	29.45
750	1.06524	1.06218	1.06012	1.05748	1.05494	1.05235	1.04976	1.04715	29.53
752	1.06818	1.06512	1.06306	1.06047	1.05788	1.05527	1.05265	1.05003	29.61
754	1.07106	1.06847	1.06588	1.06330	1.06071	1.05812	1.05553	1.05289	29.69
756	1.07394	1.07135	1.06876	1.06618	1.06359	1.06197	1.05835	1.05571	29.76
758	1.07682	1.07423	1.07165	1.06906	1.06647	1.06386	1.06124	1.05859	29.84
760	1.07971	1.07712	1.07453	1.07191	1.06929	1.06668	1.06406	1.06141	29.92
762	1.08259	1.08050	1.07741	1.07480	1.07218	1.06956	1.06694	1.06430	30.00
764	1.08547	1.08288	1.08029	1.07768	1.07506	1.07244	1.06982	1.06715	30 08
766	1.08841	1.08580	1.08318	1.08056	1.07794	1.07530	1.07265	1.06997	30.16
768	1.09129	1.08868	1.08606	1.08344	1.08082	1.07818	1.07553	1.07285	30.24
770	1.09418	1.09156	1.08894	1.08630	1.08365	1.08100	1.07835	1.07567	30.31
°F	59.0°	59.9°	60.8°	61.7°	62.6°	63.5°	64.4°	65.3°	

[a] Calcd from 1.976 = wt of 1 L of CO_2 at 0°C, 760 mm pressure, and 41° latitude. Formula given by W. Parr, *J. Am. Chem. Soc.*, 31, 237 (1909).

43.027 *Correction factors for gasometric determination of carbon dioxide* —Continued.

°C.	19.0°	19.5°	20.0°	20.5°	21.0°	21.5°	22.0°	22.5°	
mm.									*inches*
700	0.97335	0.97085	0.96835	0.96564	0.96294	0.96023	0.95753	0.95509	27.56
702	0.97618	0.97368	0.97118	0.96850	0.96582	0.96311	0.96041	0.95794	27.64
704	0.97906	0.97653	0.97400	0.97132	0.96865	0.96597	0.96329	0.96082	27.72
706	0.98188	0.97938	0.97688	0.97420	0.97153	0.96888	0.96624	0.96371	27.80
708	0.98476	0.98224	0.97971	0.97703	0.97435	0.97173	0.96912	0.96656	27.87
710	0.98759	0.98506	0.98253	0.97988	0.97724	0.97459	0.97195	0.96938	27.95
712	0.99041	0.98788	0.98535	0.98273	0.98012	0.97747	0.97483	0.97227	28.03
714	0.99329	0.99073	0.98818	0.98556	0.98294	0.98032	0.97771	0.97512	28.11
716	0.99612	0.99358	0.99106	0.98844	0.98582	0.98323	0.98065	0.97800	28.19
718	0.99900	0.99644	0.99388	0.99126	0.98865	0.98606	0.98348	0.98083	28.27
720	1.00182	0.99925	0.99671	0.99412	0.99153	0.98894	0.98636	0.98371	28.35
722	1.00465	1.00209	0.99953	0.99694	0.99435	0.99176	0.98918	0.98653	28.43
724	1.00753	1.00497	1.00241	0.99982	0.99724	0.99462	0.99200	0.98932	28.50
726	1.01035	1.00779	1.00524	1.00265	1.00006	1.99746	0.99483	0.99215	28.58
728	1.01324	1.01065	1.00806	1.00547	1.00288	1.00027	0.99765	0.99497	28.66
730	1.01606	1.01347	1.01088	1.00829	1.00571	1.00306	1.00041	1.99781	28.74
732	1.01888	1.01629	1.01371	1.01112	1.00853	1.00588	1.00324	1.00056	28.82
734	1.02176	1.01919	1.01659	1.01497	1.01135	1.00870	1.00606	1.00338	28.90
736	1.02459	1.02200	1.01941	1.01679	1.01418	1.01153	1.00888	1.00620	28.98
738	1.02747	1.02486	1 02224	1.01962	1.01700	1.01435	1.01171	1.00900	29.06
740	1.03029	1.02768	1.02506	1.02244	1.01982	1.01717	1.01453	1.01182	29.13
742	1.03318	1.03056	1.02794	1.02529	1.02265	1.02000	1.01735	1.01464	29.21
744	1.03600	1.03338	1.03076	1.02811	1.02547	1.02279	1.02212	1.01752	29.29
746	1.03888	1.03624	1.03359	1.03094	1.02829	1.02561	1.02294	1.02024	29.37
748	1.04171	1.03906	1.03641	1.03376	1.03112	1.02844	1.02576	1.02306	29.45
750	1.04453	1.04189	1.03924	1.03659	1.03394	1.03126	1.02859	1.02589	29.53
752	1.04741	1.04477	1.04212	1.03944	1.03676	1.03408	1.03141	1.02868	29.61
754	1.05024	1.04759	1.04494	1.04226	1.03959	1.03691	1.03424	1.03150	29.69
756	1.05306	1.05041	1.04776	1.04508	1.04241	1.03973	1.03706	1.03433	29.76
758	1.05594	1.05330	1.05065	1.04797	1.04529	1.04259	1.03988	1.03715	29.84
760	1.05876	1.05612	1.05347	1.05079	1.04812	1.04539	1.04265	1.03992	29.92
762	1.06165	1.05897	1.05629	1.05361	1.05094	1.04821	1.04547	1.04274	30.00
764	1.06447	1.06179	1.05912	1.05644	1.05376	1.05103	1.04829	1.04556	30.08
766	1.06729	1.06462	1.06194	1.05926	1.05659	1.05386	1.05112	1.04839	30.16
768	1.07018	1.06750	1.06482	1.06212	1.05941	1.05668	1.05394	1.05118	30.24
770	1.07300	1.07032	1.06765	1.06424	1.06224	1.05950	1.05676	1.05400	30.31
°F	66.2°	67.1°	68.0°	68.9°	69.8°	70.7°	71.6°	72.5°	

Correction factors for gasometric determination of carbon dioxide —Continued. 43.027

°C.	23.0°	23.5°	24.0°	24.5°	25.0°	25.5°	26.0°	26.5°	
mm.									*inches*
700	0.95265	0.95020	0.94776	0.94508	0.94241	0.93973	0.93706	0.93432	27.56
702	0.95547	0.95303	0.95059	0.94788	0.94518	0.94250	0.93982	0.93708	27.64
704	0.95835	0.95585	0.95335	0.95067	0.94800	0.94532	0.94265	0.93988	27.72
706	0.96118	0.95865	0.95612	0.95344	0.95076	0.94808	0.94541	0.94267	27.80
708	0.96400	0.96147	0.95894	0.95626	0.95359	0.95088	0.94818	0.94544	27.87
710	0.96682	0.96429	0.96176	0.95905	0.95635	0.95364	0.95094	0.94820	27.95
712	0.96971	0.96712	0.96453	0.96182	0.95912	0.95644	0.95376	0.95100	28.03
714	0.97253	0.96991	0.96729	0.96461	0.96194	0.95923	0.95653	0.95376	28.11
716	0.97535	0.97273	0.97012	0.96741	0.96471	0.96200	0.95929	0.95655	28.19
718	0.97818	0.97556	0.97294	0.97023	0.96753	0.96482	0.96212	0.95935	28.27
720	0.98106	0.97838	0.97571	0.97300	0.97029	0.96758	0.96488	0.96213	28.35
722	0.98388	0.98120	0.97853	0.97582	0.97312	0.97038	0.96765	0.96488	28.43
724	0.98665	0.98397	0.98129	0.97858	0.97588	0.97314	0.97041	0.96764	28.50
726	0.98947	0.98679	0.98412	0.98141	0.97871	0.97594	0.97318	0.97041	28.58
728	0.99229	0.98961	0.98694	0.98420	0.98147	0.97870	0.97594	0.97319	28.66
730	0.99512	0.99241	0.98971	0.98697	0.98424	0.98147	0.97871	0.97594	28.74
732	0.99788	0.99517	0.99247	0.98973	0.98700	0.98423	0.98147	0.97871	28.82
734	0.00071	0.99799	0.99529	0.99255	0.98982	0.98705	0.98429	0.98165	28.90
736	1.00353	1.00083	0.99812	0.99538	0.99265	0.98985	0.98706	0.98426	28.98
738	1.00629	1.00359	1.00088	0.99815	0.99541	0.99261	0.98982	0.98703	29.06
740	1.00912	1.00643	1.00371	1.00095	0.99818	0.99538	0.99259	0.98976	29.13
742	1.01194	1.00923	1.00653	1.00377	1.00100	0.99820	0.99541	0.99258	29.21
744	1.01471	1.01200	1.00929	1.00643	1.00376	1.00097	0.99818	0.99535	29.29
746	1.01753	1.01482	1.01212	1.00936	1.00659	1.00376	1.00094	0.99809	29.37
748	1.02035	1.01762	1.01488	1.01212	1.00935	1.00653	1.00371	1.00088	29.45
750	1.02318	1.02045	1.01771	1.01492	1.01212	1.00936	1.00659	1.00370	29.53
752	1.02594	1.02321	1.02047	1.01771	1.01494	1.01211	1.00929	1.00644	29.61
754	1.02876	1.02603	1.02329	1.02050	1.01771	1.01483	1.01206	1.00921	29.69
756	1.03159	1.02883	1.02606	1.02326	1.02047	1.01764	1.01482	1.01197	29.76
758	1.03441	1.03165	1.02888	1.02608	1.02329	1.02047	1.01765	1.01477	29.84
760	1.03718	1.03442	1.03165	1.02886	1.02606	1.02323	1.02041	1.01753	29.92
762	1.04000	1.03724	1.03447	1.03164	1.02882	1.02600	1.02318	1.02030	30.00
764	1.04282	1.04003	1.03723	1.03444	1.03165	1.02880	1.02594	1.02306	30.08
766	1.04565	1.04285	1.04005	1.03723	1.03441	1.03156	1.02871	1.02583	30.16
768	1.04841	1.04562	1.04282	1.04003	1.03724	1.03435	1.03147	1.02859	30.24
770	1.05123	1.04844	1.04564	1.04282	1.04000	1.03712	1.03424	1.03136	30.31
°F.	73.4°	74.3°	75.2°	76.1°	77.0°	77.9°	78.8°	79.7°	

43.027 *Correction factors for gasometric determination of carbon dioxide* —Continued.

°C	27.0°	27.5°	28.0°	28.5°	29.0°	29.5°	30.0°	30.5°	
mm.									*inches*
700	0.93159	0.92885	0.92612	0.92332	0.92053	0.91773	0.91494	0.91203	27.56
702	0.93435	0.92161	0.92888	0.92608	0.92329	0.92047	0.91765	0.91476	27.64
704	0.93712	0.93438	0.93165	0.92882	0.92600	0.92320	0.92041	0.91750	27.72
706	0.93994	0.93717	0.93441	0.93158	0.92876	0.92594	0.92312	0.92024	27.80
708	0.94271	0.93994	0.93718	0.93435	0.93153	0.92870	0.92588	0.92297	27.87
710	0.94547	0.94267	0.93988	0.93706	0.93424	0.93141	0.92859	0.92567	27.95
712	0.94824	0.94544	0.94265	0.93982	0.93700	9.93414	0.93129	0.92841	28.03
714	0.95100	0.94820	0.94541	0.94258	0.93976	0.93691	0.93406	0.93115	28.11
716	0.95382	0.95100	0.94818	0.94535	0.94253	0.93964	0.93676	0.93388	28.19
718	0.95659	0.95376	0.95094	0.94809	0.94524	0.94238	0.93953	0.93662	28.27
720	0.95939	0.95655	0.95371	0.95085	0.94800	0.94512	0.94224	0.93932	28.35
722	0.96212	0.95929	0.95647	0.95361	0.95076	0.94788	0.94500	0.94209	28.43
724	0.96488	0.96206	0.95924	0.95638	0.95353	0.95062	0.94771	0.94479	28.50
726	0.96765	0.96482	0.96200	0.95912	0.95624	0.95332	0.95041	0.94750	28.58
728	0.97041	0.96758	0.96476	0.96188	0.95900	0.95609	0.95318	0.95026	28.66
730	0.97318	0.97036	0.96753	0.96464	0.96176	0.95885	0.95594	0.95300	28.74
732	0.97594	0.97309	0.97024	0.96735	0.96447	0.96156	0.95865	0.95578	28.82
734	0.97871	0.97585	0.97300	0.97012	0.96724	0.96429	0.96135	0.95844	28.90
736	0.98147	0.97861	0.97576	0.97288	0.97000	0.96706	0.96412	0.96118	28.98
738	0.98424	0.98138	0.97853	0.97564	0.97276	0.96982	0.96688	0.96394	29.06
740	0.98694	0.98409	0.98124	0.97835	0.97547	0.97253	0.96959	0.96665	29.13
742	0.98976	0.98691	0.98406	0.98115	0.97824	0.97529	0.97235	0.96941	29.21
744	0.99253	0.98967	0.98682	0.98391	0.98100	0.97806	0.97512	0.97215	29.29
746	0.99529	0.99241	0.98953	0.98662	0.98371	0.98076	0.97782	0.97485	29.37
748	0.99806	0.99517	0.99229	0.98938	0.98647	0.98353	0.98059	0.97762	29.45
750	1.00082	0.99796	0.99506	0.99215	0.98924	0.98626	0.98329	0.98032	29.53
752	1.00359	1.00071	0.99782	0.99491	0.99200	0.98903	0.98606	0.98306	29.61
754	1.00635	1.00342	1.00059	0.99738	0.99471	0.99173	0.98876	0.98579	29.69
756	1.00912	1.00624	1.00335	1.00041	0.99747	0.99450	0.99153	0.98853	29.76
758	1.01188	1.00900	1.00612	1.00318	1.00024	0.99724	0.99429	0.99129	29.84
760	1.01465	1.01174	1.00882	1.00588	1.00294	0.99995	0.99700	0.99400	29.92
762	1.01741	1.01450	1.01159	1.00865	1.00571	1.00274	0.99976	0.99673	30.00
764	1.02018	1.01727	1.01435	1.01141	1.00847	1.00547	1.00247	0.99948	30.08
766	1.02294	1.02003	1.01712	1.01418	1.01124	1.00824	1.00524	1.00221	30.16
768	1.02571	1.02280	1.01988	1.01611	1.01394	1.01094	1.00794	1.00491	30.24
770	1.02847	1.02556	1.02265	1.01968	1.01671	1.01371	1.01071	1.00768	30.31
°F	80.6°	81.5°	82.4°	83.3°	84.2°	85.1°	86.0°	86.9°	

Correction factors for gasometric determination of carbon dioxide —Concluded. 43.027

°C	31.0°	31.5°	32.0°	32.5°	33.0°	33.5°	34.0°	34.5°	35.0°	
mm.										inches
700	0.90912	0.90620	0.90329	0.90082	0.89735	0.89432	0.89129	0.88821	0.88512	27.56
702	0.91188	0.90894	0.90600	0.90303	0.90006	0.89703	0.89400	0.89091	0.88782	27.64
704	0.91459	0.91165	0.90871	0.90576	0.90282	0.89976	0.89671	0.89362	0.89053	27.72
706	0.91735	0.91441	0.91147	0.90847	0.90547	0.90241	0.89935	0.89627	0.89318	27.80
708	0.92006	0.91712	0.91418	0.91118	0.90818	0.90512	0.90206	0.89897	0.89588	27.87
710	0.92276	0.91982	0.91688	0.91388	0.91088	0.90782	0.90476	0.90168	0.89859	27.95
712	0.92553	0.92256	0.91959	0.91659	0.91359	0.91053	0.90747	0.90438	0.90129	28.03
714	0.92824	0.92529	0.92235	0.91932	0.91629	0.91323	0.91018	0.90706	0.90394	28.11
716	0.93100	0.92803	0.92506	0.92203	0.91900	0.91594	0.91288	0.90976	0.90665	28.19
718	0.93371	0.93078	0.92776	0.92474	0.92171	0.91865	0.91559	0.91247	0.90935	28.27
720	0.93641	0.93344	0.93047	0.92744	0.92441	0.92135	0.91829	0.91517	0.91206	28.35
722	0.93918	0.93618	0.93318	0.93015	0.92712	0.92412	0.92100	0.91785	0.91471	28.43
724	0.94188	0.93897	0.93606	0.93294	0.92982	0.92676	0.92371	0.92056	0.91741	28.50
726	0.94459	0.94159	0.93859	0.93556	0.93253	0.92944	0.92635	0.92323	0.92012	28.58
728	0.94735	0.94435	0.94135	0.93830	9.93544	0.93215	0.92906	0.92591	0.92276	28.66
730	0.95006	0.94706	0.94406	0.94103	0.93800	0.93488	0.93176	0.92861	0.92547	28.74
732	0.95282	0.94979	0.94676	0.94373	0.94071	0.93759	0.93447	0.93132	0.92818	28.82
734	0.95553	0.95250	0.94947	0.94644	0.94341	0.94034	0.93718	0.93403	0.93088	28.90
736	0.95824	0.95521	0.95218	0.94915	0.94612	0.94300	0.93988	0.93670	0.93353	28.98
738	0.96100	0.95797	0.95494	0.95188	0.94882	0.94570	0.94259	0.93941	0.93624	29.06
740	0.96371	0.96068	0.95765	0.95459	0.95153	0.94841	0.94529	0.94211	0.93894	29.13
742	0.96647	0.96341	0.96035	0.95730	0.95424	0.95112	0.94800	0.94482	0.94165	29.21
744	0.96918	0.96615	0.96312	0.96003	0.95694	0.95382	0.95071	0.94750	0.94429	29.29
746	0.97188	0.96885	0.96582	0.96273	0.95965	0.95653	0.95341	0.95020	0.94700	29.37
748	0.97465	0.97159	0.96853	0.96544	0.96235	0.95925	0.95606	0.95288	0.94971	29.45
750	0.97735	0.97429	0.97124	0.96815	0.96506	0.96191	0.95876	0.95558	0.95241	29.53
752	0.98006	0.97703	0.97400	0.97088	0.96776	0.96461	0.96147	0.95826	0.95506	29.61
754	0.98282	0.97976	0.97671	0.97359	0.97047	0.96732	0.96418	0.96097	0.95776	29.69
756	0.98553	0.98247	0.97941	0.97629	0.97318	0.97003	0.96688	0.96367	0.96047	29.76
958	0.98829	0.98521	0.98212	0.97900	0.97588	0.97273	0.96959	0.96638	0.96318	29.84
760	0.99100	0.98794	0.98488	0.98176	0.97865	0.97547	0.97229	0.96908	0.96588	29.92
762	0.99371	0.99065	0.98759	0.98443	0.98135	0.97817	0.97500	0.97176	0.96853	30.00
764	0.99647	0.99338	0.99029	0.98717	0.98406	0.98088	0.97771	0.97447	0.97124	30.08
766	0.99918	0.99609	0.99300	0.98988	0.98676	0.98356	0.98053	0.97714	0.97394	30.16
768	1.00188	0.99880	0.99571	0.99259	0.98947	0.98629	0.98312	0.97986	0.97659	30.24
770	1.00465	1.00156	0.99847	0.99532	0.99218	0.98897	0.98576	0.98252	0.97929	30.31
°F	87.8°	88.7°	89.6°	90.5°	91.4°	92.3°	93.2°	94.1°	95.0°	

43.028 *Progressive accumulation of radium emanation*[a]

$I_t = I_0(1 - e^{-\lambda t})$; $\lambda = 0.1801$ days^{-1}; $I_0 = 1$

Hours	0 days	Δ	1 day	Δ	2 days	Δ	3 days	Δ	4 days	Δ	5 days	Δ	6 days	Δ	7 days	Δ
0	.0000	75	.1648	63	.3025	52	.4175	44	.5136	36	.5937	31	.6607	25	.7166	22
1	.0075	74	.1711	62	.3077	52	.4219	43	.5172	36	.5968	30	.6632	26	.7188	20
2	.0149	74	.1773	61	.3129	52	.4262	43	.5208	36	.5998	30	.6658	24	.7208	21
3	.0223	73	.1834	62	.3181	51	.4305	42	.5244	35	.6028	29	.6682	25	.7229	21
4	.0296	72	.1896	60	.3232	50	.4347	42	.5279	35	.6057	30	.6707	25	.7250	21
5	.0368	72	.1956	60	.3282	50	.4390	42	.5314	36	.6087	29	.6732	24	.7271	20
6	.0440	72	.2016	60	.3332	50	.4432	41	.5350	34	.6116	29	.6756	25	.7291	21
7	.0512	71	.2076	59	.3382	50	.4473	41	.5384	35	.6145	29	.6781	24	.7311	20
8	.0583	70	.2135	59	.3432	49	.4514	42	.5419	34	.6174	29	.6805	24	.7332	19
9	.0653	70	.2194	58	.3481	49	.4556	40	.5453	34	.6203	28	.6829	23	.7351	20
10	.0723	69	.2252	58	.3530	48	.4596	41	.5487	34	.6231	28	.6852	24	.7371	20
11	.0792	69	.2310	58	.3578	48	.4637	40	.5521	33	.6259	28	.6876	23	.7391	20
12	.0861	69	.2368	57	.3626	48	.4677	40	.5554	34	.6287	28	.6899	23	.7410	20
13	.0930	68	.2425	57	.3674	47	.4717	39	.5588	33	.6315	27	.6922	23	.7430	19
14	.0998	67	.2482	56	.3721	47	.4756	39	.5621	32	.6342	28	.6945	23	.7449	19
15	.1065	67	.2538	56	.3768	46	.4795	39	.5653	33	.6370	27	.6968	23	.7468	19
16	.1132	66	.2594	55	.3814	47	.4834	39	.5686	32	.6397	27	.6991	23	.7487	19
17	.1198	66	.2649	55	.3861	46	.4873	38	.5718	32	.6424	27	.7014	22	.7506	18
18	.1264	65	.2704	54	.3907	45	.4911	38	.5750	32	.6451	26	.7036	22	.7524	19
19	.1329	65	.2758	55	.3952	46	.4949	38	.5782	31	.6477	27	.7058	22	.7543	18
20	.1394	64	.2813	53	.3998	44	.4987	37	.5813	32	.6504	26	.7080	22	.7561	19
21	.1458	64	.2866	54	.4042	45	.5024	38	.5845	31	.6530	26	.7102	22	.7580	18
22	.1522	64	.2920	53	.4087	44	.5062	37	.5876	31	.6556	25	.7124	21	.7598	18
23	.1586	62	.2973	52	.4131	44	.5099	37	.5907	30	.6581	26	.7145	21	.7616	18

Hours	8 days	Δ	9 days	Δ	10 days	Δ	11 days	Δ	12 days	Δ	13 days	Δ	14 days	Δ	15 days	Δ
0	.7634	17	.8024	14	.8349	13	.8622	10	.8849	8	.9038	8	.9197	6	.9329	5
1	.7651	18	.8038	15	.8362	12	.8632	10	.8857	9	.9046	7	.9203	6	.9334	5
2	.7669	17	.8053	15	.8374	12	.8642	10	.8866	8	.9053	7	.9209	6	.9339	5
3	.7686	18	.8068	14	.8386	12	.8652	10	.8874	9	.9060	7	.9215	6	.9344	5
4	.7704	17	.8082	14	.8398	12	.8662	10	.8883	8	.9067	7	.9221	6	.9349	5
5	.7721	17	.8096	15	.8410	12	.8672	10	.8891	8	.9074	7	.9227	5	.9354	5
6	.7738	17	.8111	14	.8422	12	.8682	10	.8899	9	.9081	7	.9232	6	.9359	5
7	.7755	16	.8125	15	.8434	12	.8692	10	.8908	8	.9088	7	.9238	6	.9364	4
8	.7771	17	.8139	14	.8446	11	.8702	10	.8916	8	.9095	6	.9244	6	.9368	5
9	.7788	17	.8153	13	.8457	12	.8712	9	.8924	8	.9101	7	.9250	5	.9373	5
10	.7805	16	.8166	14	.8469	11	.8721	10	.8932	8	.9108	7	.9255	6	.9378	4
11	.7821	16	.8180	14	.8480	12	.8731	9	.8940	8	.9115	6	.9261	5	.9382	5
12	.7837	17	.8194	13	.8492	11	.8740	10	.8948	8	.9121	7	.9266	6	.9387	5
13	.7854	16	.8207	14	.8503	11	.8750	9	.8956	8	.9128	6	.9272	5	.9392	4
14	.7870	16	.8221	13	.8514	11	.8759	9	.8964	7	.9134	7	.9277	5	.9396	5
15	.7886	15	.8234	13	.8525	11	.8768	9	.8971	8	.9141	6	.9282	6	.9401	4
16	.7901	16	.8247	13	.8536	11	.8777	10	.8979	8	.9147	7	.9288	5	.9405	5
17	.7917	15	.8260	13	.8547	11	.8787	9	.8987	7	.9154	6	.9293	5	.9410	4
18	.7932	16	.8273	13	.8558	11	.8796	9	.8994	8	.9160	6	.9298	6	.9414	4
19	.7948	15	.8286	13	.8569	11	.8805	9	.9002	7	.9166	7	.9304	5	.9418	5
20	.7963	16	.8299	13	.8580	10	.8814	8	.9009	8	.9173	6	.9309	5	.9423	4
21	.7979	15	.8312	12	.8590	11	.8822	9	.9017	7	.9179	6	.9314	5	.9427	5
22	.7994	15	.8324	13	.8601	10	.8831	9	.9024	7	.9185	6	.9319	5	.9432	4
23	.8009	15	.8337	12	.8611	11	.8840	9	.9031	7	.9191	6	.9324	5	.9436	4

[a] *Univ. of Missouri Bull. Eng. Expt. Sta. Series* 23, Vol. 24, No. 26, 85 (1923).

Progressive accumulation of radium emanation—Concluded. 43.028

Hours	16 days	Δ	17 days	Δ	18 days	Δ	19 days	Δ	20 days	Δ	21 days	Δ	22 days	Δ	23 days	Δ
0	.9440	9	.9533	7	.9610	6	.9674	5	.9728	4	.9773	3	.9810	3	.9842	2
2	.9449	8	.9540	6	.9616	5	.9679	5	.9732	4	.9776	4	.9813	3	.9844	2
4	.9457	8	.9546	7	.9621	6	.9684	5	.9736	4	.9780	3	.9816	3	.9846	3
6	.9465	8	.9553	7	.9627	6	.9689	4	.9740	4	.9783	3	.9819	3	.9849	2
8	.9473	8	.9560	6	.9633	5	.9693	5	.9744	4	.9786	3	.9822	2	.9851	2
10	.9481	8	.9566	7	.9638	5	.9698	4	.9748	4	.9789	3	.9824	3	.9853	2
12	.9489	7	.9573	6	.9643	6	.9702	5	.9752	3	.9792	4	.9827	2	.9855	2
14	.9496	8	.9579	7	.9649	5	.9707	4	.9755	4	.9796	3	.9829	3	.9857	2
16	.9504	7	.9586	6	.9654	5	.9711	4	.9759	3	.9799	3	.9832	2	.9859	2
18	.9511	7	.9592	6	.9659	5	.9715	5	.9762	4	.9802	3	.9834	3	.9861	2
20	.9518	8	.9598	6	.9664	5	.9720	4	.9766	3	.9805	3	.9837	2	.9863	2
22	.9526	7	.9604	6	.9669	5	.9724	4	.9769	4	.9808	2	.9839	3	.9865	2

Hours	24 days	Δ	25 days	Δ	26 days	Δ	27 days	Δ	28 days	Δ	29 days	Δ	30 days	Δ	31 days	Δ
0	.9867	4	.9889	4	.9908	2	.9923	2	.9936	1	.9947	1	.9955	2	.9963	1
4	.9871	4	.9893	3	.9910	3	.9925	2	.9937	2	.9948	2	.9957	1	.9964	1
8	.9875	4	.9896	3	.9913	3	.9927	2	.9939	2	.9950	1	.9958	1	.9965	1
12	.9879	3	.9899	3	.9916	2	.9929	3	.9941	2	.9951	2	.9959	1	.9966	1
16	.9882	4	.9902	3	.9918	2	.9932	2	.9943	2	.9953	1	.9960	2	.9967	1
20	.9886	3	.9905	3	.9920	3	.9934	2	.9945	2	.9954	1	.9962	1	.9968	1

43.029 *Optical-crystallographic properties of some crystalline drugs*[a]

COMPOUND	α	β	γ	OPTIC SIGN	EXTINCTION	ELONGATION	2 V	REMARKS
Antibiotics								
Penicillin sodium G	1.550	1.609	1.620	−	p	+	large	n_β common.
Penicillin potassium G	1.550	—	1.603	−	p	+		Elongated rectangular plates
Penicillin potassium O	1.545	—	1.593		p	+		
Penicillin procaine G	1.545	1.570	1.685	+	p, i	±		n_β common.
Penicillin *l*-ephedrine G	1.575	—	1.610		p	−		
Penicillin *l*-ephenamine G	1.583	1.590	1.648	+	i		very small	Bx. ac. figs.
Penicillin benzathine G	1.523	1.622	1.630	−	p	+	very small	n_α and n_γ common.
Penicillin G hydriodide diethylaminoethyl ester	1.601	1.608	1.632	+	p	−	moderate	
Penicillin dibenzylamine G	1.567	—	1.613		p	+		
Penicillin hydrabamine G	1.556	ca 1.590	1.619		p, i	−		
Penicillin chloroprocaine O	1.541	1.585	1.656	+	p	±	very large	
Dihydrostreptomycin sulfate	1.552	1.558	1.566	+	p, i		89°	Ext. angle = 18°.
Dihydrostreptomycin hydrochloride	1.522	1.548	1.566	−	p	+	80°	
Chlortetracycline hydrochloride	1.635	1.706	1.730	−	p, s		59°	
Oxytetracycline dihydrate	1.634	1.646	>1.700	+	p		28°	
Oxytetracycline hydrochloride	1.546	1.635	1.730	+	p, i	+	very large	Op. ax. figs. common.
Tetracycline trihydrate	1.538	1.646	sl > 1.787	+	p, i		large	Occasional op. ax. figs.
Tetracycline hydrochloride	1.603	1.685	1.714	−	p		large	Bx. ac. and op. ax. figs.
Chloramphenicol	1.523	1.608	1.659	−	p	−	70–80°	
Chloramphenicol palmitate	1.527	—	1.569		p	±		
Gramicidin	1.541	ca 1.553	1.573	+				
Tyrocidine hydrochloride	1.553	—	1.584		p	+		
Erythromycin dihydrate	1.512	1.523	1.532	−	p	−	84°	
Erythromycin oxalate dihydrate	1.484	1.492	1.516	+	p	+	60°	
Erythromycin ethyl carbonate	1.496	1.506	1.510	−	p	+	moderate	Op. ax. figs. common.
Erythromycin glucoheptonate	1.506	—	1.528		p	+		
Erythromycin hydriodide hydrate	1.528	1.536	1.550	+	p	+	75°	
Carbomycin	1.474	1.484	1.513	+	p	±		
Fumagillin	1.518	ca 1.572	>1.780	+	p, i		small	

[a] Abbreviations: p = parallel; s = symmetrical; i = inclined; n = index; Bx.ac. = acute bisectrix; Op.ax. = optic axis; fig. = figure; sl = slightly.

COMPOUND	α	β	γ	OPTIC SIGN	EXTINCTION	ELONGATION	2 V	REMARKS
			Antihistamines					
Antergan* Hydrochloride	1.587	1.635	1.734	+				Short prisms. Inclined figs.
Anthallan* Hydrochloride	1.505	1.585	1.617		p	−		Small rods & irregular fragments. No figs.
Bromothen Hydrochloride	1.617	1.654	1.734		i	+		Very small rods. Inclined figs. occas'l.
Chlorcyclizine Monohydrochloride (Di-Paralene* Hydrochloride)	1.590	1.610	1.665	+				Thin platy fragments. Op. ax. figs. common.
Chlorcyclizine Dihydrochloride (Perazil* Dihydrochloride)	1.610	1.660	1.665	−	p	+	very small	Short rods & thin 6-sided plates. Op. ax. figs. common.
Chlorprophenpyridamine Maleate (Chlor-Trimeton* Maleate)	1.533	n, 1.668	sl < 1.734					Box-like prisms & irregular fragments. Figs. infrequent
Chlorothen Citrate (Tagathen*)	1.583	1.603	1.645					Minute plates & shreds. Op. ax. figs. rare.
Chlorothen Hydrochloride	1.553	1.625	>1.734					Massive fragments, some rectangular. Op. ax. figs. occas'l.
Dimenhydrinate (Dramamine*) (Unsatisfactory for optical-crystallographic study.)								Platy material & rods.
Diphenhydramine Hydrochloride (Benadryl* Hydrochloride)	1.602	1.625	1.630	−	p	−		6-sided plates.
p-Fluorobenzyl (D.P.E.) Hydrochloride	1.585	1.600	1.668	+	p	−	large	Rods & square plates. Op. ax. figs. occas'l.
Linadryl Hydrochloride	1.577	1.631	1.672	−	p, i	+		Elongated 6-sided & irregular fragments. Figs. rare.
Methaphenilene Hydrochloride (Diatrine* Hydrochloride)	1.604	1.675	1.733	−				Elongated 6-sided rods with obtuse ends. Op. ax. figs. frequent.
Methapyrilene Hydrochloride (Histadyl*, Thenylene* Hydrochloride)	1.588	1.654	>1.695 but <1.734	−				Thick hexagonal plates. Inclined op. ax. figs. frequent.
Phenergan* Hydrobromide	1.667	1.675	>1.733	+			small	Massive prisms. Elongated or short & stubby.
Phenergan* Hydrochloride	1.617	1.691	1.733	−	p	+		Rods & irregular fragments.
Prophenpyridamine Maleate (Trimeton* Maleate)	1.548	1.574	1.665	+	p	+	small	Rods & plates.
Pyrathiazine (Pyrrolazote* Hydrochloride)	1.690		1.737					Stout prismatic forms. No figs.
Pyrilamine Maleate (Unsatisfactory for optical-crystallographic study.)								Rods & irregular fragments.

* Trademarked name.

43.029 *Optical-crystallographic properties*—Continued.

COMPOUND	α	β	γ	OPTIC SIGN	EXTINCTION	ELONGATION	2 V	REMARKS
Thenfadil Hydrochloride	1.590		1.680				large	Square plates & stubby prisms. Inclined figs. common.
Thonzylamine Hydrochloride (Neohetramine* hydrochloride)	1.612	1.679	1.691	–	p	+		Rods & platy material.
Tripelennamine Hydrochloride (Pyribenzamine* hydrochloride)	1.580	1.655	1.705	–				Rectangular plates & prisms from water. Op. ax. figs. common.
			Barbiturates					
Allylbarbituric acid (5-allyl-5-isobutylbarbituric acid)	1.508	n_1 1.521	1.577	+	p	+	moderate	Rods and plates.
Alphenal (5-allyl-5-phenylbarbituric acid)	1.551	1.578	1.645	+	p	–	67°	Op. ax. fig. common.
Amobarbital (5-isoamyl-5-ethylbarbituric acid)	1.467	—	1.539		p	+		Both n's common.
Amobarbital Sodium	(n) 1.505	—	—	Isotropic				
Aprobarbital (5-allyl-5-isopropylbarbituric acid)	1.520	1.581	1.600	–	i		moderate	Rods.
Barbital (diethylbarbituric acid)	1.445	1.548	1.580					All n's common.
Barbital Sodium	1.512	1.532	1.615			+	40°	
Butallylonal [5-(2-bromallyl)-5-sec-butylbarbituric acid]	1.524	1.577	1.603	–	p	+	moderate	Rosettes of tiny rods and blades. Bx. ac. figs. occas'l.
Butethal (5-butyl-5-ethylbarbituric acid)	1.454	1.518	1.556	–	i		large	Rods & needles. Op. ax. and bx. ac. figs. common.
Cyclobarbital (Phanodorn*) (5-ethyl-5-cyclohexenylbarbituric acid)	1.515	1.546	1.621	+		±	69°	Bx. ac. & bx. ob. figs. common.
Cyclopal (5-cyclopentenyl-5-allylbarbituric acid)	1.520	1.575	1.626	–		–	85°	Bx. ac. fig. common.
Dial* (5,5-diallylbarbituric acid)	1.516	1.572	1.625	–	s		large	Op. ax. fig. common.
Hexethal (Ortal*) (5-ethyl-5-*n*-hexylbarbituric acid)	1.473	1.519	1.549	–		–	76°	Bx. ac. fig. common.
Hexobarbital (5-cyclohexenyl-1,5-dimethylbarbituric acid)	1.546	1.608	1.634	–	p	+	64°	Bx. ac. & op. ax. figs. common.
Mephobarbital (5-ethyl-1-methyl-5-phenylbarbituric acid)	1.594	1.610	1.651	+	p	–	65°	Bx. ac. fig. common.
Pentobarbital [5-ethyl-5-(1-methylbutyl) barbituric acid]	1.465	—	1.565	–	i		very large	——
Pentobarbital Sodium	1.477	—	1.523					——
Phenobarbital (5-ethyl-5-phenylbarbituric acid)	1.557	1.620	1.667		p	–		β very common.

Optical-crystallographic properties—Continued.

COMPOUND	α	β	γ	OPTIC SIGN	EXTINCTION	ELONGATION	2 V	REMARKS
Phenobarbital Sodium (unstable)								
Probarbital (5-ethyl-5-isopropylbarbituric acid)	1.477	1.573	1.624	−	i	+	73°	Rods.
Probarbital Sodium	1.532	—	1.629		p	+		Rods & needles.
Secobarbital (Seconal*) [5-allyl-5-(1-methylbutyl) barbituric acid]	1.487	1.557	1.563	−	p	+	31°	Bx. ac. fig. common.
Secobarbital Sodium	1.490	n_i 1.500	1.525					n_i & γ common.
Sigmodal* [5-*s*-amyl-5-β-bromallylbarbituric acid]	1.519	1.583	1.634	−		+	80°	Bx. ac. fig. common.
Thiopental [5-ethyl-5-(1-methylbutyl) thiobarbituric acid]	1.534	1.634	—	−	i		40–45°	Lamellar.
Vinbarbital [5-ethyl-5-(1-methyl-1-butenyl) barbituric acid]	1.506	1.544	1.672	+	p	−	61°	Bx. ac. fig. common.
Sulfonamides								
Sulfacetamide	1.559	1.564	1.727	+	s		21°	
Sulfadiazine	1.596	1.675	1.830	+	p, i	±	76°	
Sulfadiazine[c]	1.615	1.663	>1.734		p	±		Rods.
Sulfaguanidine	1.606	1.663	1.734					Op. ax. fig.
Sulfaguanidine monohydrate	1.586	1.649	1.731	+	p, i	±	86°	
Sulfallantoin* (sulfanilamide + allantoin-addition product)	1.513	1.590	>1.690 <1.733					Op. ax. fig.
Sulfamerazine	1.568	1.657	1.687	−	p	±	58°	Bx. ac. fig. all n's common.
Sulfamerazine[c]	1.587		1.675					
Sulfamethazine	1.584	1.623	>1.778					Rods.
Sulfamidazole* (sulfanilamide + sulfathiazole-double crystal[b])	1.661	1.678	>1.733	+	p	−	small	Bx. ac. fig.
Sulfanilamide phase B (anhyd.)	1.555	1.672	1.85	+	p	−		Stable form coml prepns.
Sulfanilamide Hydrochloride	1.540	1.655	1.690		p	−		Rods.
Sulfapyridine, Phase I	1.670	1.736	1.813	+	p, i	±	88°	Tabular to equant; stable form coml prepns.

[b] Equimolecular proportions.
[c] The second set of optical properties in each case represents intermediate data which are quite commonly found in some commercial samples. They probably represent an hydrous form or merely a different common orientation of the crystal.

43.029 *Optical-crystallographic properties*—Continued.

COMPOUND	α	β	γ	OPTIC SIGN	EXTINCTION	ELONGATION	2 V	REMARKS
Sulfapyridine	1.680	1.733	>1.733					Op. ax. fig.
Sulfapyridine Sodium monohydrate	1.590		1.700		p	—		
Succinylsulfathiazole (Sulfasuxidine*)	1.578	1.676	1.710	—	i		58°	Rods.
Sulfathiazole, Phase I	1.674	1.685	>1.733	+			small	α & β common.
Sulfathiazole, Phase II	1.598	1.741	1.780	—	p, i	±	52°	Lath shaped.
Sulfathiazole[c]	1.695	n_i1.733	>1.733					
Sulfathiazole Sodium sesquihydrate	1.596		1.621					
Sulfisoxazole (Gantrisin*)	1.605	1.642	1.697	+	p	±	large	Plates & rods.
			Sympathomimetic Amines					
d-Amphetamine Hydrochloride	1.560	1.592	1.622	+	p, i	±	very large	Large plates & rods. Op. ax. figs. occas'l.
dl-Amphetamine Hydrochloride	1.508	1.582	1.611		p	—		Rods and plates.
d-Amphetamine Phosphate, dibasic	1.546	1.583	1.664	+	p	±	moderate	Plates with truncated corners.
dl-Amphetamine Phosphate, dibasic	1.549	1.589	1.665	+			large	Small platy crystals. Bx. ac. figs. common.
d-Amphetamine Sulfate (Dexedrine* Sulfate)	1.501	1.545	1.608	+		—	small	6–8-sided plates.
dl-Amphetamine Sulfate (Benzedrine* Sulfate)	1.520	1.531	1.614	+		—	very sm.	
Benzazoline Hydrochloride (Priscoline* Hydrochloride)	1.586	1.604	1.703	+	i			6-sided plates. Inclined op. ax. figs. common.
d-Desoxyephedrine Hydrochloride (Methamphetamine Hydrochloride)	1.530	1.537	1.615	+				Irregular fragments. Op. ax. figs. frequent.
dl-Desoxyephedrine Hydrochloride	1.535		1.620		p	—		Small 6-sided platy or rod-like crystals. No figs.
dl-Ephedrine Hydrochloride (Racephedrine Hydrochloride)	1.570	1.608	1.630	—				Irregular fragments. Occasional op. ax. figs.
l-Ephedrine Hydrochloride	1.530	1.603	1.638	—	p	—	70°	Elongated prisms & rods.
l-Ephedrine Sulfate	1.540	1.565	1.587	+	p		large	6-sided plates & rods.
dl-Epinephrine	1.551	1.599	1.736	+	p	—	moderate	Thin, blade-like, 6-sided crystals in rosettes. Bx. ac. figs. common.
l-Epinephrine	1.551	1.599	1.736	+	p	—	moderate	Rosettes of thin, blade-like, 6-sided crystals. Bx. ac. figs. common.

43.029 *Optical-crystallographic properties*—Concluded.

COMPOUND	α	β	γ	OPTIC SIGN	EXTINCTION	ELONGATION	2 V	REMARKS
Hydroxyamphetamine Hydrobromide (Paredrine* Hydrobromide)	1.560	1.680	1.734	−				Irregular fragments. Inclined op. ax. fig. frequent.
Naphazoline Nitrate	1.560	1.619	>1.740	+	s, i			6-sided plates & irregular fragments. Bx. ac. figs. common.
Phenylpropylmethylamine Hydrochloride (Vonedrine* Hydrochloride)	1.577		1.603		p	−		Small rod-like fragments. No figs.
Sepriphen Hydrochloride	1.507	1.604	1.668	−	p	±		Rectangular rods. Bx. ob. figs. common.
dl-Synephrine* base (Desoxyepinephrine)	1.546	1.604	1.725	+	s, i		large	Platy crystals, often diamond-shaped. Op. ax. figs. common.
Synephrine* Hydrochloride	1.549	1.605	1.664	+	p, i		large	Large plates. Bx. ac. figs. frequent
dl-Synephrine* (+) Tartrate (Neutral Salt)	1.516	n_i1.620	1.689	+	i	+	large	Rods & plates. Partial op. ax. figs. common.
Tuaminoheptane Sulfate (Tuamine* Sulfate)	1.458_ω		1.468_ϵ	+	p			Irregular-shaped plates & fibrous flakes. Figs. frequent.
Veritol Sulfate (Isodrine Sulfate)	1.516	1.552	1.645	+	s	+		Rhombohedral or 6-sided plates.

43.030 *Determinative table of refractive indices for drugs, arranged according to ascending value of the lowest index*[a]

α	β	γ	COMPOUND
			Antibiotics
1.474	1.484	1.513	Carbomycin
1.484	1.492	1.516	Erythromycin oxalate dihydrate
1.496	1.506	1.510	Erythromycin ethyl carbonate
1.506	——	1.528	Erythromycin glucoheptonate
1.512	1.523	1.532	Erythromycin dihydrate
1.518	ca 1.572	>1.780	Fumagillin
1.522	1.548	1.566	Dihydrostreptomycin hydrochloride
1.523	1.608	1.659	Chloramphenicol
1.523	1.622	1.630	Penicillin benzathine G
1.527	——	1.569	Chloramphenicol palmitate
1.528	1.536	1.550	Erythromycin hydriodide hydrate
1.538	1.646	sl >1.787	Tetracycline trihydrate
1.541	ca 1.553	1.573	Gramicidin
1.541	1.585	1.656	Penicillin chloroprocaine O
1.545	——	1.593	Penicillin potassium O
1.545	1.570	1.685	Penicillin procaine G
1.546	1.635	1.730	Oxytetracycline hydrochloride
1.550	1.609	1.620	Penicillin sodium G
1.550	——	1.603	Penicillin potassium G
1.552	1.558	1.566	Dihydrostreptomycin sulfate
1.553	——	1.584	Tyrocidine hydrochloride
1.556	ca 1.590	1.619	Penicillin hydrabamine G
1.567	——	1.613	Penicillin dibenzylamine G
1.575	——	1.610	Penicillin *l*-ephedrine G
1.583	1.590	1.648	Penicillin *l*-ephenamine G
1.601	1.608	1.632	Penicillin G hydriodide diethylaminoethyl ester
1.603	1.685	1.714	Tetracycline hydrochloride
1.634	1.646	>1.700	Oxytetracycline dihydrate
1.635	1.706	1.730	Chlortetracycline hydrochloride

[a] See 43.029 for symbols.

Determinative table of refractive indices for drugs—Continued. 43.030

α	β	γ	COMPOUND
		Antihistamines	
1.505	1.585	1.617	Anthallan* Hydrochloride
1.533	n_i1.668	sl <1.735	Chlorphenpyridamine Maleate (Chlor-Trimeton* Maleate)
1.548	1.574	1.665	Prophenpyridamine Maleate (Trimeton* Maleate)
1.553	1.625	>1.734	Chlorothen Hydrochloride
1.577	1.631	1.672	Linadryl Hydrochloride
1.580	1.655	1.705	Tripelennamine Hydrochloride (Pyribenzamine* Hydrochloride)
1.583	1.603	1.645	Chlorothen Citrate (Tagathen*)
1.585	1.600	1.668	*p*-Fluorobenzyl (D.P.E.) Hydrochloride
1.587	1.635	1.734	Antergan* Hydrochloride
1.588	1.654	>1.695 but <1.734	Methapyrilene Hydrochloride (Histadyl*, Thenylene* Hydrochloride)
1.590	1.610	1.665	Chlorcyclizine Monohydrochloride (Di-Paralene* Hydrochloride)
1.590		1.680	Thenfadil Hydrochloride
1.602	1.625	1.630	Diphenhydramine Hydrochloride (Benadryl* Hydrochloride)
1.604	1.675	1.733	Methaphenilene Hydrochloride (Diatrine* Hydrochloride)
1.610	1.660	1.665	Chlorcyclizine Dihydrochloride (Perazil* Dihydrochloride)
1.612	1.679	1.691	Thonzylamine Hydrochloride (Neohetramine* Hydrochloride)
1.617	1.654	1.734	Bromothen Hydrochloride
1.617	1.691	1.733	Phenergan* Hydrochloride
1.667	1.675	>1.733	Phenergan* Hydrobromide
1.690		1.737	Pyrathiazine (Pyrrolazote* Hydrochloride)
		Barbiturates	
1.445	1.548	1.580	Barbital (Diethylbarbituric acid)
1.454	1.518	1.556	Butethal (Neonal*)
1.465		1.565	Pentobarbital (Nembutal*)
1.467		1.539	Amobarbital (Amytal*)
1.473	1.519	1.549	Hexethal (Ortal*)
1.477	1.573	1.624	Probarbital (Ipral*)
1.477		1.523	Pentobarbital Sodium (Nembutal* Sodium)
1.487	1.557	1.563	Secobarbital (Seconal*)
1.490	n_i1.500	1.525	Secobarbital Sodium (Seconal* Sodium)
(n)1.505	Isotropic		Amobarbital Sodium (Amytal* Sodium)
1.506	1.544	1.672	Vinbarbital (Delvinal*)
1.508	n_i1.521	1.577	Allylbarbituric Acid (Sandoptal*)
1.512	1.532	1.615	Barbital Sodium
1.515	1.546	1.621	Cyclobarbital (Phanodorn*)
1.516	1.572	1.625	Diallylbarbituric Acid (Dial*)

[a] *See* 43.029 for symbols.

43.030 *Determinative table of refractive indices for drugs*—Continued.

α	β	γ	COMPOUND
1.519	1.583	1.634	Sigmodal*
1.520	1.575	1.626	Cyclopal*
1.520	1.581	1.600	Aprobarbital (Alurate*)
1.524	1.577	1.603	Butallylonal (Pernoston*)
1.532	—	1.629	Probarbital Sodium (Ipral* Sodium)
1.534	1.634	—	Thiopental (Pentothal*)
1.546	1.608	1.634	Hexobarbital (Evipal*)
1.551	1.578	1.645	Alphenal
1.557	1.620	1.667	Phenobarbital (Luminal*)
1.594	1.610	1.651	Mephobarbital (Mebaral*)
			Sulfonamides
1.513	1.590	>1.690 but <1.733	Sulfallantoin*
1.540	1.655	1.690	Sulfanilamide Hydrochloride
1.555	1.672	1.85	Sulfanilamide, Phase B (anhydrous)
1.559	1.564	1.727	Sulfacetamide
1.568	1.657	1.687	Sulfamerazine
1.578	1.676	1.710	Succinylsulfathiazole (Sulfasuxidine*)
1.584	1.623	>1.778	Sulfamethazine
1.586	1.649	1.731	Sulfaguanidine Monohydrate
1.587		1.675	Sulfamerazine
1.590		1.700	Sulfapyridine Sodium Monohydrate
1.596		1.621	Sulfathiazole Sodium Sesquihydrate
1.596	1.675	1.830	Sulfadiazine
1.598	1.741	1.780	Sulfathiazole, Phase II
1.605	1.642	1.697	Sulfisoxazole (Gantrisin*)
1.606	1.663	1.734	Sulfaguanidine
1.615	1.663	>1.734	Sulfadiazine
1.661	1.678	>1.733	Sulfamidazole*
1.670	1.736	1.813	Sulfapyridine, Phase I
1.674	1.685	>1.733	Sulfathiazole, Phase I
			Sympathomimetic Amines
1.458_{ϵ}		1.468_{ϵ}	Tuaminoheptane Sulfate (Tuamine* Sulfate)
1.501	1.545	1.608	*d*-Amphetamine Sulfate (Dexedrine* Sulfate)
1.507	1.604	1.668	Supriphen Hydrochloride
1.508	1.582	1.611	*dl*-Amphetamine Hydrochloride
1.516	1.552	1.645	Veritol Sulfate (Isodrine Sulfate)
1.516	n_i 1.620	1.689	*dl*-Synephrine* (+) Tartrate (Neutral Salt)
1.520	1.531	1.614	*dl*-Amphetamine Sulfate (Benzedrine* Sulfate)
1.530	1.537	1.615	*d*-Desoxyephedrine Hydrochloride (Methamphetamine Hydrochloride)
1.530	1.603	1.638	*l*-Ephedrine Hydrochloride
1.535		1.620	*dl*-Desoxyephedrine Hydrochloride
1.540	1.565	1.587	*l*-Ephedrine Sulfate
1.546	1.583	1.664	*d*-Amphetamine Phosphate, dibasic
1.546	1.604	1.725	*dl*-Synephrine* base (Desoxyepinephrine)
1.549	1.589	1.665	*dl*-Amphetamine Phosphate, dibasic
1.549	1.605	1.664	Synephrine* Hydrochloride

Determinative table of refractive indices for drugs—Concluded. **43.030**

α	β	γ	COMPOUND
1.551	1.599	1.736	*dl*-Epinephrine
1.551	1.599	1.736	*l*-Epinephrine
1.560	1.592	1.622	*d*-Amphetamine Hydrochloride
1.560	1.619	>1.740	Naphazoline Nitrate
1.560	1.680	1.734	Hydroxyamphetamine Hydrobromide (Paredrine* Hydrobromide)
1.570	1.608	1.630	*dl*-Ephedrine Hydrochloride (Racephedrine Hydrochloride)
1.577		1.603	Phenylpropylmethylamine Hydrochloride (Vonedrine* Hydrochloride)
1.586	1.604	1.703	Benzazoline Hydrochloride (Priscoline* Hydrochloride)

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Entries will usually be found under the constituent sought rather than under the product in which it is to be determined, *e.g.*, the determination of potassium in fruits will be found under potassium rather than under fruits. Secondary determinations (*e.g.*, water-soluble ash) have been included with the primary determination (ash).